Julian Huxley
(1887-1975)

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Julian Huxley—a 1965 photograph,
by Janet Stone

Volume 254   March 6 1975

Guide to authors

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Cover Picture

The vitamin-deficient bat
See p 148

TOMORROW'S TECHNOLOGY

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Cover Picture

J S Haldane with King George V
and Queen Mary at Bristol in 1925
Asa Briggs reviews a recent book on
Haldane on p 213
[University of Bristol photograph]

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Evolutionary conservation of H-Y ('male') antigen—*S S Wachtel, G C Koo and E A Boyse* 270

Production of lymphoid tumours in hamsters by direct implantation of normal human leukocytes—*I Miyoshi, I Kubonishi, T Hayashi, S Abe, H Uchida, T Tsubota and T Tanaka* 272

Matters arising

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The Radi

Cover Picture
The path of a metal drop under water, page 321
Photo Paul Brierley

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Cover Picture

'The Editor' Rebecca Lucy Davies'
(then aged 3¾) impression of her
daddy But why do the arms come
out of the head? See p 416

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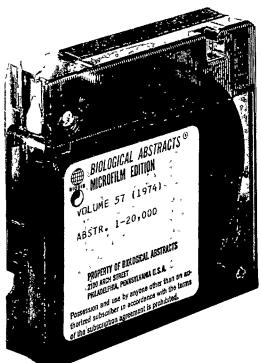
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Cover Picture

A yellow glazed vessel of archaic
bronze form (Chueh) Ch'ing dynasty
(18th century), 13 3 cms high
The Percival David Foundation of
Chinese Art
Analysing Chinese glazes—p 501

Cover Picture

Endeavour's seamen gather ice in the Antarctic, January 9, 1773 Captain Cook wrote 'The Melting of the Ice is a little tideous, otherwise this is the most expeditious way of Watering I ever met with' Antarctic science on pp 569 583 591 Antarctic policy on p 546

nature

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Cover Picture

Acernaspis suffeita (Lamont) A
schizochroal-eyed trilobite from the
Pentland Hills near Edinburgh
Silurian × 5 See page 663

Volume 254   April 24, 1975

Pure Che
SCIENCE COLLEGE

Organochlorine Insecticides

Persistent Organic Pollutants
edited by F. Moriarty

May/June 1975, xii + 302 pp, £7 90/$21 00   0 12 506750 X

This book represents an attempt to indicate the general principles which will help to predict and control events, and to pinpoint the important gaps in our knowledge   While the range of topics covered is wide from analytical chemistry to possible legal and economic restraints, with the major emphasis being placed on biological effects and responses — attention is restricted to persistent organic pollutants, and in particular to the organochlorine insecticides which are by far the most studied   The ideas that are proposed will however apply to a far wider range of pollution problems

Fluid-bed Heat Transfer

Gas-fluidized bed behaviour and its influence on bed thermal properties
J. S. M. Botterill

May/June 1975, x + 300 pp, £9 00/$23 75   0 12 118750 0

Gas-fluidized beds have extremely desirable heat transfer properties, though in industry they have been comparatively unexploited for these specific advantages   This volume, based on the author's past and present work, describes the range of behaviour encountered in gas-fluidized systems and shows how this results in the beds' advantageous thermal properties

The work, dealing with a subject in which there is still scope for experimental investigation, will provide much-needed information for engineers and applied scientists working in the chemical and fuel industries, the metallurgical industry and areas of mechanical engineering   At the same time it will prove of value to those in the food processing industry and to all with an interest in fluid particle systems

Scandium

Its Occurrence, Chemistry, Physics, Metallurgy, Biology and Technology
K. A. Gschneidner, G. T. Horovitz (Editor),
G. A. Nelson, H. H. Schock and D. H. Youngblood

April/May 1975, approx 600 pp., approx. £12 00 /$31 75   0 12 355850.6

This encyclopedic work about one chemical element, compiled by experts in various scientific fields, has a considerable scope ranging from simple chemical reactions to metallurgy and applications   The volume brings together important research developments and original data from various sources, and will also have great value as a reference work where a critical analysis of published literature has been made   The book is interdisciplinary, and is therefore aimed at a wide audience including research workers in rare earth metals, postgraduate students, specialist chemists, physicists, metallurgists, biologists and geologists

Structure of Metallic Catalysts

J. R. Anderson

May/June 1975, x + 468 pp, £12 80/$33 75   0 12 057150 1

This is the first book which attempts a coherent account of the structure of metallic catalysts, in particular the author tries to relate general preparative methods and ultimate structure to the chemistry and physics of the materials concerned

Contents Introduction to metals and catalysts   Support materials   Massive metal catalysts   Dispersed metal catalysts   Structure and properties of small metal particles   Measurement techniques surface area, particle size and pore structure   Measurement techniques   surface composition and structure   Appendix 1   Data for metals   Appendix 2   Illustrative recipes for the preparation of metallic catalysts, Subject index

Effects of Interferon on Cells, Viruses and the Immune System

edited by A. Geraldes

June/July, xii + 662 pp, approx. £17 00/$43 25 0 12 280650 6

This book is based on the proceedings of the Gulbenkian Institute Symposium on interferon, to collate information on fundamental problems regarding mechanisms of induction and action and their regulation   The book discusses many major breakthroughs, and reviews the present state of interferon research   A considerable amount of emphasis is placed on the action of interferon on cell metabolism, its depressing influence on the immune system, and its subsequent effect on grafts   Other progress is represented by contributions on the antiviral effect of Sepharose-bount interferon, interferon messenger RNA, and the genetic regulation of the interferon system   Focusing attention on aspects of a new phase of interferon research, this volume will excite interest among biologists, biochemists, virologists, immunologists and those involved in medical research.

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nature

March 6, 1975

Who makes the decision on life and death?

Thou shalt not kill; yet needst not strive,

Officiously to keep alive.

Arthur Hugh Clough's witty Victorian Ten Commandments for the comfortable sometimes have a very modern ring to them—none more so than the sixth when put in the context of modern medicine. Last week BBC television gave us two looks at spina bifida; the first was an hour-long documentary of great distinction which managed at once to educate, to convey a message of hope, to present the caution of scientists in an understanding light and to provoke on the question of whether severely handicapped babies should be left to die. The second took up the running on this last issue in the programme "Controversy", and Dr John Lorber, who is one of many pediatricians who give parents the chance to withhold treatment in bad cases, was confronted with two doctors and a theologian who believed this option should not be given, and an audience which seemed (at least after editing) to divide roughly equally; it's tantalising that the producers of "Controversy" don't ask for a vote, even after the formal ending.

It was no great surprise that the parents at the debate provided the most emotional and memorable contributions, nor that the lady from the National Secular Society should have said what she did about terminating more lives. Somewhat suprisingly, the theologian Professor Gordon Dunstan, stated the case for preserving life in muted terms: let them die and you reduce the capacity for heroism and the incentive to medical science. For the rest, the debate rested fairly inevitably on the technical data and philosophies of different doctors.

And yet one thing did emerge with remarkable clarity —that it is the doctor, not the parents who makes the decision whether the child lives or dies. If the doctor believes severe cases should be allowed to die relatively peacefully then he will be capable of convincing almost all his clients to follow this course. If, on the other hand, he believes severe cases deserve every attention that medical science can bestow, he will be able to take parents along with him in that direction.

There is nothing new in all this. In the matter of birth control and abortion, sophisticated women have recognised for many years the necessity for pre-selecting the person whom they consult.

The reason that the doctor has so much power in the decision-making process is not simply that he has immense knowledge where his client has none—after all, the decision that the parents have to make is one in which technical knowledge is only one ingredient. Rather,

the parents must be only too aware that if the doctor expresses a view one way and they choose to ignore his opinion, they have to live not only with their own decision but with the doctor himself for months if not years. It is presumably for the same reasons that people don't more often pick arguments with teachers, bosses and dentists.

Dr Lorber has broken valuable new ground by publicising a new option for parents so courageously (although the point was made in the programme that doctors do occasionally confront comparable problems in other circumstances). Many have, and will, object to the granting of the option on grounds that there are possibilities that the child could live a happy life, but it is striking how little public uproar there has been on grounds of morality; it must be that the obvious detached sincerity with which many parents have elected not to support their child's life, and the frequent damage to family life—even culminating in suicide—that spina bifida can bring, prevents some of the more conventional sanctity-of-life responses that one would otherwise expect. But how do we ensure that it is truly an option, and for that matter that the option is available to those that need it, regardless of the hospital area in which they happen to live?

However carefully the doctor may present both sides of the case it is inevitable that his clients are strongly influenced by the slightest hint of a preference one way or the other, and the choice which was originally theirs has subtly been surrendered to the doctor. In many cases this may be no bad thing, but this does not necessarily justify continuing a procedure in which some clients may eventually look back in some puzzlement at how they reached a particular decision. Offering a second opinion does not seem a good safety net; few seek it, and although doctors may claim this is because clients can come to their own conclusions, it may equally indicate tentativeness of clients in the face of medical men.

An idea worth consideration is that the medical profession, or even some organisation outside the profession, should provide a new sort of service to doctors and clients in such circumstances. Someone without any involvement in an individual case should be deputed by the doctor to present the options to the client, proffer advice if requested and then convey the decision to the doctor. In this way not only would it be clearer that the decision has been made by those who must ultimately bear the responsibilities, but also there would be assurance that the range of options discussed reflects all medical possibilities. ∎

Huxley remembered

SIR JULIAN HUXLEY, FRS, one of the best known scientific personalities of our day, died in London on February 14 at the age of 87.

Huxley was a King's Scholar at Eton and went on to Balliol, Oxford, where he became Brackenbury Scholar and, from 1910–12, a lecturer in zoology. As a undergraduate, he was also awarded the Newdigate Prize for Poetry. At 25, he became Professor of Zoology at the Rice Institute in Houston, but at the outbreak of war returned home and joined the Army Intelligence Corps, serving at the Italian front when the armistice was signed. He returned to Oxford in 1919 as a Fellow of New College and Senior Demonstrator in Zoology, and in 1925 was appointed Professor of Zoology at King's College, London. He also held the office of Fullerian Professor of Physiology at the Royal Institution from 1926–28, but resigned both scientific posts to devote more time to writing and research. His association with the Zoological Society of London as Secretary began in 1935 and lasted until 1942, during which time he helped to develop Whipsnade Zoo. His lifelong interest in Africa resulted in his being a member of the committee of Lord Hailey's 'African Survey' from 1933–38, and a member of the 1944 Committee on Higher Education in West Africa.

Because of his experience and powerful interest in the problems of social evolution and education, the British government persuaded him to become the first Director-General of UNESCO in 1946.

His many publications on animal behaviour, evolution and genetics are too numerous to mention. His *Problems of Relative Growth* (1932) was a turning point in the study of differential growth of parts of the body, and *Evolution, the Modern Synthesis* (1948) remains the most comprehensive modern work on this subject. But beguiled audiences of the BBC's 'Brains Trust' programme will remember him best as one of the original panellists, and particularly during the Second World War. Huxley also lent his support to many other institutions—the British Humanist Association, the Family Planning Association, the Nature Conservancy, and so on.

Recognition of his work came from all sectors. Among many were the Kalinga Prize (1953) for popular science writing, the Darwin Medal of the Royal Society (1957) for contributions to the study of .evolution, and a Knighthood in 1958. •   □

JULIAN HUXLEY was only a decade or so older, but he was one of my 'heroes' from the time (1921) when I was starting research in chemical embryology. I never knew him really well and never spent very much time with him, but he was always there as a kind of leading figure "amidst the encircling gloom"; and occasionally our lives impinged strongly on each other. In the 1920s he was perhaps best known for his work at Oxford on the hybrid control of amphibian metamorphosis. This was outside my field, as I never worked in classical endocrinology, but when the 1930s came I was extremely interested in Julian's book on heterogony (or heterauxesis, as it afterwards came to be called) *Problems of Relative Growth*. Together with Georges Teissier in France I spent a good deal of time in applying these conceptions to the relative growth of the chemical constituents of animal bodies. This turned out to be one of those lines of of investigation which, though interesting in themselves, do not lead immediately to anything further. Quite different was the situation with Julian's *Elements of Experimental Embryology*, which he wrote with G.R. de Beer in 1934. This was at the beginning of the story of embryonic induction: based on

the fundamental experiments of Spemann and his collaborators, it set the stage for a vast amount of work on the 'morphogenetic hormones'. Terminologies change, and organisers today might be called 'semantophore macro-molecules', but although such great advances have been made in the study of the hereditary 'books of instructions', the nucleic acid chains, it seems that still today it has not been possible to identify the molecules which pass from cell to cell and determine specific differentiation destinies. Doubtless messenger RNA is in the picture somewhere. In any case Huxley and de Beer was a stirring overture to a field of experimental morphology still of the highest importance and fascination today.

But neither Julian Huxley nor I were spirits circumscribed by the walls of the laboratory. Both of us were interested in philosophy, religion, artistic creation, history and literature; both of us, like Charles Sherrington and many other scientists, were moved to write poems from time to time. We must retrace our steps, for *Religion without Revelation* came out in 1927: while my *Sceptical Biologist* was 1929. Of course our standpoints were rather different, though both in a way syn-

cretistic Julian upheld the family tradition of agnosticism and what came to be called 'humanism', while I was for accepting the practice of all the forms of human experience while not granting absolute validity to any of them Consequently I retained liturgical religion and was content to be described as a left-wing Christian socialist —but all this never came between us, and on a great many matters we saw closely eye to eye It was rather like an experience I once had with Fred Hoyle The Cambridge Union proposed to _debate that "wherever science advances religion recedes", and he was to be the proposer, while I was cast as the opponent So we had tea together, and concluded, after carefully looking into it, that there was just that hair's breadth of difference between us as to make the affair possible It was rather like that with Julian and me

But politics kept on breaking in Over a couple of decades, centering on the 1930s, the "Younger Scientists" became a label as political as the "Young Turks" had been in an earlier time Here the essential crux was the relations of science and society, what really were they in the liberal democratic, bourgeois, capitalist society that we all knew, what had they been in the societies of the long-past world, and what could they be imagined to be in socialist society, whether nascent as at that time in the USSR, or more ideally foreseeable in future socialist societies? This movement of the 1930s was the lineal ancestor of such bodies as the British Society for Social Responsibility in Science today, with the big difference that present leading men, such as Joseph Rotblat and Stephen Rose, are contending with a world of nuclear power and nuclear weapons in which the problems of pollution have come much more into the foreground than they were in the 1930s But in those days you had a very powerful group, of which perhaps Julian Huxley and J B S Haldane were the oldest, including Conrad Waddington, Desmond Bernal, Lancelot Hogben, Dorothy Crowfoot and Hymen Levy Other scientists were radical, though not on the left, such as Cyril Darlington and John Baker, while others yet again, like Patrick Blackett and Solly Zuckerman, so phrased their sociological beliefs as not to lose touch with the 'corridors of power' and the establishment, thus retaining a chance of influencing national policy All these 'elements', *quorum pars minimus fui,* met together from time to time in the "Quots and Tots", a dining club in London which was only extinguished by the Second World War Needless to say, it took its name from the phrase *quot homines tot sententiae,* but in fact throughout the 1930s there was a real consensus of agreement among the "Younger Scientists" Their *pronunciamientos* constantly enraged the older generation entrenched in Burlington House, where so strong was the prejudice against scientists doing anything other than science, that I was reduced (though I do not think Julian ever was) to adopting a pseudonym for some of my extra-scientific writings Some may remember that in allusion to certain extremely distinguished officers of the Royal Society, the "Younger Scientists" were accused of riding roughshod over hill and dale

Subjects like animal behaviour and bird-watching were always closed books to me a side of Julian that I never knew Our orbits came into the closest conjunction, however, after the foundation of UNESCO in 1946 I always thought it was Ellen Wilkinson, not R A Butler as has recently been said, who nominated Julian Huxley as the first Director-General, at any rate I was far away in China at the time I had been one of those who throughout the last years of the war had made great agitation to get science included with education and culture in this new specialised agency of the United Nations And it was therefore the greatest of pleasures to get a cable from Julian (I forget whether it was in Chungking or Nanking) asking me to return at once to head up the Natural Sciences Division I duly came, we established ourselves in a large, gaunt, empty house of ambassadorial character in Belgrave Square, and the recruitment of staff went merrily on The UNESCO years brought me into very close touch with Julian and that was the time I got to know him best In those days UNESCO officials were not bound down by too much red tape, as happened later, for example when the Naples Marine Biological Station was about to close down, as its seawater pumps had worn out, a couple of telephone calls with Stafford Cripps elicited that there were plenty of American surplus pumps on the quayside in that very city, and these could be used to keep things going Or again, as soon as we knew that the Poles had lost, during the war, all their printing machines capable of setting up mathematical equations, Julian and I by a couple of telephone calls could get some new machines moving on their way to Warsaw

The UNESCO period, fortunately, found Julian Huxley in one of his periods of most frenetic energy and activity Our daily conferences were unforgettable, with André Thomas Thomas for culture, and Kuo Yu-Shou for education Dear Dr Kuo, I had known him a long time before when he was Commissioner of Education in Szechuan Province Those were the days when we formulated between us many a policy for which afterwards, indeed for the rest of his life, Julian Huxley did great propaganda work— the liquidation of illiteracy, the control of population increases, and the reproduction of great classical spiritual books in many languages other than those in which they were originally written Some of these enterprises have prospered remarkably through the years—for example the international understanding inculcated by the *UNESCO Courier,* which appears all over the world in many languages, or again the UNESCO *History of the World* Julian used to maintain that this was originally my idea, but I feel quite certain that it was his, and all I did was to back it up through thick and thin at various meetings, including those of the Executive composed of the delegates of all the member states This work in many volumes must now be in school libraries all over the world and really may be said to be fairly free from nationalistic prejudices Thomas, Kuo and I were very close to Julian, and only Thomas carried on for any length of time after the first change of Director-General Besides, there were not only the meetings but also very companionable walks and meals on Sundays in the forests of Fontainbleau and Rambouillet where we could compare notes *ad lib*

Finally, in a last act, as it were, Julian and I enormously enjoyed investigating Amerindian archaeology on the occasion of the Second General Conference of UNESCO in Mexico City Together we scoured the wonderful National Museum, visited unforgettable sites, like Teotihuacan, Xochicalco and Chichen-Itza, and sat at the feet of Mexican scholars and writers like Alfonso Caso, Miguel Covarrubias and Alberto Ruz-Lhuillier All this bore much fruit for me later on when in the fourth volume of *Science and Civilisation in China* I had to face the problem of pre-Columbian culture contacts across the Pacific from East Asia

Julian Huxley was a man for whom nothing human was foreign, one thought naturally of the phrase *nihil humanum me alienum puto* He was blessed beyond measure by his marriage with Juliette, happy essentially from beginning to end, right from the day when her father's *vignerons* took Julian to be Oexle—*le type qui a inventé les dégrés* All their friends think of her with the deepest affection, and hope that knowledge of this may moderate, even in however small a way, the sense of loss from which she, like us, is assuredly suffering ☐

Joseph Needham

Huxley : the presence of the 'S' in UNESCO is largely due to him

JULIAN HUXLEY was the leading experimental biologist of his day at a time and in a place in which the ever more detailed validation of the concept of evolution was still thought of as the principal task of biology The supplanting of comparative anatomy by what everybody came to call 'experimental biology' is a fascinating episode in the history of ideas In the early days of the Darwinian revolution the elucidation of homologies and the working out of family trees had the same kind of appeal and confident self importance as molecular biology has today, and I know from personal conversations with him that E S Goodrich, the former Linacre Professor of Zoology and Comparative Anatomy at Oxford and the only British comparative anatomist of the same stature as Gegenbaur and van Wijhe, still regarded himself even quite late in life as a torchbearer and adventurous pioneer of the great new doctrine

But, alas, comparative anatomy became an abuse and an impediment to progress, just as comparative physiology became in the next quarter of a century and as molecular biology will surely become in 10 or 20 years (for I foresee clearly a time when, regardless of what major problems in biology remain to be attended to, the sequencing of a protein will still be regarded as an intrinsically meritorious activity which will be taken to represent the courageous holding aloft of the banner of what was once a great revolution in biological ideas)

Because of his intellectual vitality and the great compass of his interest and understanding Huxley rightly earned for himself the position of acknowledged leader of the newer biology Certainly nobody since has made contributions of comparable magnitude to fields so diverse as ethology (his papers on the courtship behaviour of the great crested grebe are acknowledged classics), physiological genetics, developmental physiology including the study of allometry or differential growth, and ecology, especially in relation to speciation

And then again, Huxley was, as his grandfather had been, a great expositor of the notion of evolution, although sometimes his ideas on the subject were felt by his juniors, rightly or wrongly, to be wrong-headed—mainly because Huxley never really mastered the modern population-dynamical approach to evolution theory, and so great was Huxley's enthusiasm for the idea of evolution that he came in his later years to treat evolutionism as a sort of secular religion

Huxley was a great tutor in the old

Oxford style, a man who, because he loved it, chose to teach the whole of his subject instead of teaching only the parts of it that interested him and for the remainder farming out his students to specialists in other fields The influence of a really good tutor lasts through many generations of pupils, pupils' pupils and so on It is pleasing therefore to reflect that through a lineage which can be worked out with names and dates in detail, Julian's son Francis was his own great grand-pupil (The lineage is Julian Huxley–Gavin de Beer–J Z Young–P B Medawar–Francis Huxley)

With these qualities of character it is not at all surprising that Huxley was kind and helpful to the young, for although people in his position are bombarded from all quarters of the Earth by manuscripts of which the authors profess to seek their recipient's candid opinion, Huxley bore with this kind of imposition very handsomely and often made the time to answer his correspondents at length—sometimes in his own handwriting

I do not know and cannot imagine any scale of evaluation of scientific merit along which Huxley would not stand out as one of the foremost biologists of the 20th century ☐

Peter Medawar

THE contribution that Julian Huxley made to the work of UNESCO in the field of science was remarkable and, in several respects, decisive

In the first place, he fought to ensure that science was given a place among the organisation's concerns on an equal footing with education and culture Thirty years ago, when the conference convened to adopt the Constitution of UNESCO was about to meet in London in November 1945, the issue was still in the balance On one side, the followers of the classical humanist tradition thought it better to deal only with education and culture, since they both centred on the preservation and development of moral values On the other, some scientists took the view that science had become far too complex as an intellectual and social activity, and too important on account of its practical applications, to be only one of the fields of competence of an institution, they looked for a distinct organisation solely and totally concerned with science Huxley was one of those who checked these separatist tendencies The presence of the 'S' in UNESCO is largely due to him

Had the organisation restricted itself to dealing with education and culture,

it would have been no doubt easier to manage, with probably a greater immediate efficiency But none of the difficulties encountered outweighs, in my opinion, the paramount advantages and significance of the existence of an organisation which embraces, through the inter-relationship of its various fields of competence, the comprehensive unity of the minds as a whole

Once this inter-relationship of education, science and culture was adopted as the fundamental principle of UNESCO, no one could have been better qualified than Huxley, with his manifold gifts, varied experience and wide-ranging earlier ventures, to provide the framework of the new organisation's programme And that was the main task to which he devoted the best of his energies and abilities, as Secretary-General of the Preparatory Commission (1946) and thereafter as the first Director-General (1946–48)

In the field of natural sciences, with the help of Joseph Needham, he devoted special attention to the restoration and reshaping, in collaboration with the International Council of Scientific Unions (ICSU), of the international scientific community which had been shattered by the war But Huxley was no less interested in the social sciences, which he thought should have a part to play in all sectors of UNESCO's activities Thus, he put an ethnologist in charge of the first 'fundamental education' pilot project in Haiti His efforts to bring together scientists, educationists and users of the mass media in a wide-ranging movement for the modernisation of science teaching and, more generally, for the popularisation of science, provides another instance of his multidisciplinary approach to problems

Science for him was not merely a body of knowledge and skills, it was the most advanced form of culture, the very basis of all values This feeling of the close kinship of science and culture suggested to him the idea of a *History of the Scientific and Cultural Development of Mankind* He put the proposal to the General Conference at its first session in 1946 and succeeded—though not without difficulty—in securing its adoption And after he had relinquished his functions as Director-General he played a very active part in carrying it out as Vice-President of the International Commission which had the editorial responsibility for the work

One final aspect of his contribution to UNESCO's science programme which deserves special mention is that he wished the organisation to foster among both governments and the

general public a better awareness of certain major issues affecting the future of mankind With his biological background and his evolutionist approach, he was admirably equipped to understand such problems and put them in perspective In matters concerning the preservation of nature and its ecological balances, the proper management of the resources of the biosphere, the quantitative and qualitative implications of population growth, and the problems of human settlements, the views and the programmes that Julian Huxley recommended to UNESCO were a quarter of a century ahead of the ideas of the time They showed a remarkable perception of the true mission of international organisations, which we are now just beginning to discover That mission is not simply to help member states in solving their particular problems It is above all to bring the people of the world to understand the vital problems of mankind as a whole, which require for their solution a sense of togetherness and the joint efforts of the community of nations □

René Maheu

By the death of Sir Julian Huxley we lose not only a distinguished scientist but a man with whom it was as easy to talk of art or literature as of biology I met him first, I think, in 1921 in the exciting society at Garsington, where he and his wife were staying with Lady Ottoline Morrell

At that time Huxley was a Fellow of New College and Senior Demonstrator in Zoology at Oxford Though about as much archaeologist as biologist, I attended his course on genetics In the practical classes which accompanied it we studied the inheritance of eye colour in a small superficially shrimp-like animal, a species of *Gammarus* Chancing to make an unexpected observation which seemed to open up certain possibilities of general interest, I discussed the matter with him In a flash he saw the point and greatly extended my ideas in our subsequent talks and in our delightful excursions to Plymouth to study the animal in its natural habitat and to obtain further material for our investigations He, already a well known scientist, and I, an unknown undergraduate, at once started to research together Our first account of that work appeared in *Nature* in 1925, and we published extensively on it in the next few years And here I would stress a quality entirely characteristic of Julian Huxley When we published our major article (in the *British Journal of Experimental Biology*), giving a detailed account of our results with the conclusions to be drawn from them, it appeared not under

the names of Huxley and Ford but of Ford and Huxley It has been a lesson to me all my life to encourage and give priority to my junior colleagues

This seems to have been among the last pieces of experimental work in which Julian Huxley took part His scientific writings, extending over many years, have been of fundamental importance, his success as Secretary of the Zoological Society of London, and as Director-General of UNESCO was outstanding and needs no encomium from me I would, however, like to draw attention to his greatest and least recognised achievement

In his position, he was frequently visiting universities and scientific institutes of various kinds He would talk to those researching there and from this something unprecedented would emerge He would encounter those who had, perhaps for months or years, been devoting their time to some biological problem of which, often enough, Huxley would know very little In a short conversation he would almost invariably be able to throw a new light on it, and those who talked with him felt that his visit had been an outstanding occasion It was a contribution to science of a most unusual and unselfish kind, one which only a genius could make He obtained little recognition for it, but its cumulative effect was great and can never be assessed

It is difficult to impart an idea of Julian Huxley's friendship It can, perhaps, be comprehended in this He would always speak of a friend better behind his back than to his face few indeed deserves such a tribute □

E. B. Ford

Julian Huxley was a great teacher, and not only in the early academic phase of his career The extent of his influence on zoologists of my own and younger generations is not always realised

He was a leader of the movement which gathered so much momentum in the early 1920s, away from conventional comparative anatomy and the construction of evolutionary family trees to experimental embryology, genetics, comparative physiology and functional analysis In 1923, with Hogben, Crew and J B S Haldane, he launched the Society for Experimental Biology and its journal, then called the *British Journal of Experimental Biology*, Hogben has described the quartet as the Founding Fathers of the society —and the society has done more to guide the development of biology and of young biologists in this country in its half century of thriving expansion than any other

My association with Julian was closest in the late 1920s, when we col-

laborated with my father, H G Wells, in writing *The Science of Life* This was conceived by H G as a companion to his earlier *Outline of History,* to set down plainly and clearly "everything that an educated man—to be an educated man—ought to know about biological science We three got together in 1927 and we made a scheme that covered every division of our immense subject We worked very harmoniously throughout and, after a part publication, produced the book in 1930 " My quotations are from H G's *Experiment in Autobiography*

Those were strenuous years Julian has written in considerable detail about *The Science of Life* in the first volume of his *Memories* (1970), and told how the harmony of the three authors was occasionally obscured by superficial dissonance Most of the text was first written by Julian (who produced far the greater portion) and myself H G's functions were to edit what we wrote and to drive us on to write it There were times of special stress in 1929, when the first of the fortnightly parts were published while others were in page proof, others in galley, others in typescript and some even in the planning stage H G was never an easy man to work with, as many tempestuous episodes in his life reveal, and he could be furious when his collaborators failed to deliver their copy as soon as he wished, or wrote at much greater length than had been planned But the storms soon subsided, in Julian's words "H G lost and recovered his temper, and so did I, but on the whole the atmosphere was gay and friendly" I am sure that the violence of H G's storms was lessened, partly by his great respect for Julian's store of vivid and accurate information and partly by his realisation that he himself was being educated He had studied under Julian's grandfather at the Royal College of Science, now he was learning from the grandson how far and how excitingly the subject had evolved since those days

In any event, the book was written and widely read, appearing over the years in several editions and revisions and in several languages In Julian's own assessment, "The work was indeed an important achievement It is now out of print but its effects are still manifest in the increased space allotted to biology in the educational curriculum, and the greater interest of the general public in biological facts and their consequences " What he does not say is that the work would never have seen the light had it not been for his enthusiasm, his great abilities, and, as I have hinted, his fundamental friendliness and generosity □

G. P. Wells

international news

FUTURE historians of science may well record that a highly significant event took place in a California state park between February 24 and 27, 1975 Meeting at the Asilomar Conference Center here for three days of intense—and at times heated—discussions, 140 scientists from 16 countries agreed that strict controls should be placed on an exciting new technique which enabled genes from one organism to be transplanted directly into another

This attempt at self regulation by a group of scientists is believed to be without direct precedent, for although restrictions have been imposed on research before, they have usually resulted from concern over the uses to which science will be put, or from public pressure In this case, however, the call for controls has come from the very scientists most actively engaged in the research and it has come at the inception of a new field of study, before any known hazards have arisen

Although the genetic transfer technique promises revolutionary advances in molecular biology, the conference decided that its use should be tightly controlled because the genetically modified organisms it creates may pose direct, but unpredictable, health hazards Such concerns led a group of American biologists, headed by Paul Berg of Stanford University, to issue a call last July for a world wide suspension of research involving the technique, until the possible hazards associated with it have been evaluated (*Nature*, **250**, 175, 1974) This conference was called to try to assess the hazards and to decide under what circumstances such research should be resumed

The meeting concluded, in short, that some genetic manipulation experiments could go ahead immediately, provided fairly strict safety precautions are adopted, but that others should not be attempted until safer procedures have been developed It was also agreed that there are some types of experiments which are potentially so hazardous that they should not be carried out under any circumstances A statement of the principal conclusions reached at the meeting is now being drawn up

It was remarkable that even a statement of general principles was approve by all but a tiny handful of participants at the conference, for at times it seemed inevitable that there would be a disastrous split between those who

Berg conference favours use of weak strains

from Colin Norman, Pacific Grove

wanted to press ahead with their experiments and those who were arguing for restraint In the end, however, the rift was healed by the realisation that safer procedures could be developed very rapidly to allow virtually all the planned experiments to proceed, so that any continuation of the suspension of experiments would be short lived The nub of the matter is that gene transfer experiments could be carried out much more safely using biologically disabled microorganisms which would be unable to survive outside the laboratory, instead of the organisms that are commonly used in genetic research Fortunately, it was generally agreed that by using what one participant described as "old fashioned steam genetic engineering", such enfeebled strains could be developed and produced in quantity in a matter of weeks, so that even the most gung-ho researchers were able to accept that it would be prudent to delay some of their riskier experiments until safer organisms are available

Coming as they do from the people who will be most affected by them, the proposed controls on such experiments may seem like the classic case of the fox being set to guard the chickens—in fact, they have already been criticised as such by a group of radical scientists known as Science for the People But the recommendations are much stricter than might be expected and, if implemented, they would require expensive modifications to many laboratories hoping to conduct gene transfer experiments They are also more stringent than recommendations published last month by a committee of British scientists, chaired by Lord Ashby, which concluded that although development of safer strains of experimental organisms would be desirable, present safety precautions would probably be sufficient to allow most experiments to proceed

The technique in question, according to Sydney Brenner of the MRC Laboratory for Molecular Biology, Cambridge, "is going to generate the most

exciting period, I think, in biology, and it is going to last for at least 10 years, maybe more" It involves use of a newly discovered class of enzymes, called restriction enzymes, to snip fragments of genetic material (DNA) from one organism and splice them to the DNA of another organism, such as a virus or bacterium

The key to the technique is that some restriction enzymes sever one strand of the double-stranded DNA leaving what are known as 'sticky ends' Two DNA molecules cut by the same enzyme will have identical ends, which can be joined together and annealed into a single molecule Three types of experiment using this method of cutting and splicing together DNA fragments from different sources have either been carried out or are being contemplated The first involves insertion of 'foreign' DNA fragments into a package of DNA called a plasmid, which can replicate inside a bacterium independently of the bacterium's chromosomes A second type of experiment involves splicing fragments of foreign DNA into the DNA of viruses called bacteriophages, which attack and replicate in bacteria And a third possibility involves joining together pieces of DNA from two different viruses, to form a hybrid virus

The scientific promise of the technique is that it will allow fragments of DNA which are believed to be responsible for initiating specific chemical reactions to be inserted into a bacterium and copied every time the bacterium divides The bacterial culture would then produce large quantities of the foreign DNA fragment, which could be studied more easily The technique also offers the possibility of taking particular genes out of their normal environment and studying how they operate in the simple genetic system of a bacterium And the construction of hybrid viruses may help to shed light on why some viruses cause tumours in animals

In addition to that potentially rich scientific harvest from such genetic manipulation experiments, the technique may offer the more remote possibility of isolating genes which code for nitrogen fixation in leguminous plants and introducing them into the cells of crops such as cereals If that could be accomplished the resulting hybrid cereals would no longer need a constant supply of nitrogen fertiliser Another widely touted possibility is

that it may be possible to mass-produce the genes responsible for promoting the synthesis of insulin by growing them in bacterial cultures

But the technique also involves the possibility of significant health hazards The essence of the matter is that by combining fragments of DNA from different sources an infectious organism might be produced endowed with unpredictable biological properties By splicing genes from two viruses, for example, the host range of the virus might be extended, so that a virus which does not normally infect might inadvertently be made to do so The consequences of transferring segments of DNA from animal tumour viruses to viruses which commonly infect man, for example, could be disastrous The chief reason for concern with such experiments is that segments sliced from a molecule of DNA by a restriction enzyme contain considerable amounts of genetic material in addition to the particular gene which is being studied, and the effect of that unknown DNA cannot be predicted in advance

Another cause for concern is that the bacterium most commonly used in genetic research is a laboratory strain of *E coli*, a bacterium which is normally present in the human gut Although Dr E S Anderson, of the Enteric Reference Laboratory, Colin Dale, presented evidence suggesting that the particular strain of *E coli* used in laboratory research does not survive for long in the gut in competition with other strains, it was universally agreed that more reliable safety factors are needed

That realisation led to perhaps the most productive session at the conference—a session during which specific mutations were discussed which could be built into laboratory viruses and bacteria used for genetic research, to ensure that the organisms would be incapable of surviving outside the medium in which they were cultured

According to Waclaw Szybalski, of the University of Wisconsin, such biologically enfeebled strains could be produced "by the time we have slept off this meeting " In short, the mutations would involve making bacteriophage lambda (the most commonly used bacteriophage for genetic manipulation experiments) unable to survive at human body temperature, unable to grow the tails needed to make it infectious, and able to infect only the particular modified strain of bacteria used in the experiment *E coli* could also be equipped with genetic mutations which would make it unable to synthsise diaminopimelic acid (DAP), a constituent of cell walls, so that it would have to be provided with DAP in order to survive. *E coli* could also be made temperature-sensitive

With the promise that such disabled organisms could be produced and made widely available in a matter of weeks, much of the concern about delaying experiments, which had been voiced during the first two days of the meeting, was considerably diminished Consequently, a draft statement, drawn up by the oragnising committee and discussed on the final day, was accepted by an overwhelming majority of the participants, even thought it called for many important experiments to be put off until safer laboratory strains of bacteriophage and *E coli* are developed

The draft statement outlined three levels of safety precautions which should be accompanying various genetic manipulation experiments Only the lowest risk experiments should be carried out with existing strains of bacteriophage lambda and *E coli*, the statement suggested, but they would require safeguards such as autoclaving all cultures, safe pipetting procedures and so on Such experiments would consist of gene transfers between organisms which normally exchange genetic material—in other words, no novel genetic combinations would result from the experiment—and the insertion of genes from invertebrates and cold-blooded vertebrates into bacteria

The next level of experiment—those embodying moderate risk—would require use of biological safety cabinets, negative pressure in limited access laboratories and use of some protective clothing Estimates of how much it would cost to equip a laboratory to conduct such experiments varied widely, but it was generally reckoned that at least \$20,000–40,000 would be needed In addition because physical safeguards cannot guarantee absolute protection against the spread of biological agents, such experiments should await the development of enfeebled strains of viruses and bacteria, the statement suggested Experiments such as the splicing together of DNA from viruses, linkage of viral DNA to bacterial plasmids, and the joining of fragments of DNA from warm-blooded animals to bacterial DNA would fall into this category

The highest risk experiments which could be done would again require the use of enfeebled strains of bacteria, and they should only be conducted in special laboratories equipped with airlocks to isolate them from other areas Protective clothing should be worn, and showers would be required on entering and leaving the laboratory Examples of such experiments are the fusion of genes to bacterial DNA when the resulting organism is likely to produce an agent toxic to the host, and work on viruses such as smallpox, which present a high risk in themselves

Finally, after much debate, it was agreed that there are some types of experiments which should be ruled out altogether Although such experiments were not defined, they would include the insertion into *E coli* of the gene which specifies production of botulinus toxin There would, however, be little justification for carrying out such experiments, except to produce lethal agents for biological warfare

A statement setting out those general principles was agreed to by all but about three or four participants Among the dissenters were two Nobel prizewinners, Joshua Lederberg, of Stanford University, and James Watson, of Cold Spring Harbor, both of whom had consistently challenged some of the basic assumptions underlying the conference

Lederberg, for example, said in a statement he distributed at the conference, that "research on recombinant DNA is, in my opinion, the central way in which molecular genetics can contribute to the solution of important medical problems" and he warned repeatedly that any delays in carrying out the research should take into account the fact that important benefits may also be delayed He also suggested that regulations governing such research are likely to be frozen in place, so that it would be difficult to make them less stringent later if it turns out that the risks have been overestimated Lederberg also took exception to the classification of experiments into only three classes, pointing out that most of the contemplated studies would fall into the low or moderate risk categories, but the difference between the two is "considerable reconstruction of a laboratory"

Watson drew attention to the discrepancy between regulations being proposed for genetic manipulation studies and those governing work on tumour viruses "As someone in charge of a tumour laboratory", he said, "we are working with something I feel is instinctively more dangerous than anything I have heard here", yet the regulations governing research on tumour viruses are relatively lax "I think you should just use common sense", he said, but added that "you will have to live with the fact that somebody may sue you for \$1 million if you are careless"

Underlying much of the discussion of the need for self-imposed controls was acceptance of the fact that science has lost much of the public support that it once enjoyed, and that if regulations were not imposed from within, legislation could be anticipated which would probably turn out to be much more restrictive Whether this unprecedented attempt at self regulation will work, however, remains to be seen □

Facing Israel's water problems

from Kapai Pines, Jerusalem

IN spite of a 25-year-old development scheme for water resources, Israel is faced with a crisis which threatens to damage seriously the country's agricultural programme The Israelis' ingenuity in water management has achieved spectacular results, particularly in the cultivation of the so-called wilderness areas, but now the country has reached the point where exploitable resources are strictly limited, while consumption is growing and promises to go on growing

Israel has a uniquely uniform pressurised supply system which encompasses virtually the whole country and integrates almost all of its water resources, 95% of available natural sources are used The problem is how to expand, and even to conduct, the system in a situation of growing demand and limited supply, exacerbated by the problem of deteriorating quality

Recently, water problems have been the subject of a lively public debate, in which almost everybody is trying to participate In a memorandum submitted to the Prime Minister, Mr Rabin, the Minister of Agriculture, Mr Ozan, appeals for an urgent allotment of IL 500 million for the development of new water resources this year Mr Ozan claims that there has not been any development of such new resources for many years and that, if immediate steps are not taken it will be necessary to divert 300 million cubic metres of water from agriculture in order to meet the expanding demands of urban consumption As a result, agricultural production will shrink by 25%

Further, on February 10, almost all the Knesset (Israeli Parliament) factions united in adopting a resolution calling for water planning funds to develop new water resources while carefully utilising the existing resources to the peak of their economy and efficiency The resolution warns against pollution of underground water, Lake Kinneret (Sea of Galilee), rivers and wadies, it calls for the implementation of sea water desalination by nuclear energy

It is characteristic that the experts are divided among themselves as to the right solution In the face of almost fully utilised natural resources what are the alternatives? There are several There is, of course, the possibility of limiting the growth of consumption and adjusting it to the level of supply It is also possible, some experts claim, to increase the actual yield of the resources by using water which has not been used for various reasons so far (brackish water or sewage effluents, very deep borehole well water or flood waters in winter streams) It is also possible to produce 'artificial water' (artificial rainfall and converted saline water) An additional way is 'mining' of water from underground reservoirs, which are one-time-only reserves

As far as desalination goes, almost all experts agree that the technologies available at present are generally too costly for producing water for agricultural use Since the urban and the industrial sections have been given priority, agriculture must take the blow □

Water yield and consumption in Israel (in millions of cubic metres per annum)

(a) Water yield

Resource	Potable water Present yield capacity (1972–73)	Potable water Estimated future development (end of century)	Marginal water (brackish flood water reclamation of waters, sewage effluents) Present yield capacity	Marginal water (brackish flood water reclamation of waters, sewage effluents) Estimated future development
Lake Kinneret (Sea of Galilee)	560	150		10
Main aquifers	960	20	160	20
Floods	30	20–40	13	8
Sewage Effluents		100	26	100
Desalination	2	12		
Total	1,552	302–322	199	138

(b) Water consumption

Sector	Potable water Consumption in 1972–73	Potable water Recent annual increase	Other waters brackish, flood-water, sewage effluents
Agricultural	1,175	20	167
Domestic	285	12	
Industrial	60	5	32
Total	1,520	37	199

Mr. Wilson's deal with Russia

from Vera Rich

THE recent visit of Mr Wilson to the USSR has resulted, *inter alia*, in an impressive programme for scientific and technological cooperation between the UK and the USSR for the next decade This programme is, in fact, a conseqeunce of a number of meetings and discussions on this theme, going back as far as 1968 and culminating in the Agreement on the Development of Economic, Scientific, Technological and Industrial Cooperation signed in London on May 6, 1974

The programme envisages a wide range of activities in which scientific and technological cooperation can be "encouraged" on a "mutually advantageous basis", ranging from high temperature plasma physics to "continuous beer production", and it is difficult to assess how far any particular clause will be implemented Some subjects seem to have been included on a rather unilateral basis—it is difficult to to see, for example, what precise benefit the UK would receive from the joint study of irrigation—and others are already there as a sop to the prevailing trends of world opinion (energy-saving studies and reduction of diesel exhausts), yet others (such as astronomy) are so wide ranging that it is difficult to know precisely what cooperation is envisaged To complicate the matter further, the official programme blandly states after an impressive list of fields of cooperation that it is only "of a recommendatory nature, and is subect to modification and adjustment as and when necessary", a statement which appears to be something of an escape clause

Nevertheless, some areas of cooperation can be defined more closely A general statement of cooperation on "flameproof equipment for use in mines subject to sudden gas emissions and coal and rock bursts" seems to link up with a clause in the detailed schedule on "Cooperation to equip Soviet mining machines supplied to the United Kingdom with British-made electric equipment in accordance with British safety standards" (a somewhat surprising statement in view of the frequent *Novosti* releases on the safety of the Soviet mining industries) and the suggestion that the Soviet Union should participate "in the realisation of the programme of the National Coal Board for the modernisation and expansion of coal output" (which could raise some piquant problems with the Trade Unions) Meanwhile, in the Soviet Union, British know-how is to be used in the supply of goods ranging from "airport equipment" to "equipment for

THE Indian University Grants Commission (UGC) has decided that emphasis during the Fifth Plan will be on consolidation and strengthening of existing university departments Because of severe financial constraints, the UGC has asked all universities to curtail expenditure and limit their programmes according to its guidelines Accordingly, the universities have been asked to formulate detailed programmes for development of existing teaching and research departments, introduction of new areas of specialisation in existing departments and setting up of individual departments, measures such as updating and modernisation of courses, and giving specific orientation to research activities, improvements in library facilities and services and general amenities for students and staff, such as study centres, hostels, residential quarters and health centres The universities have also been asked to see that, while they plan new programmes, those initiated during earlier Plan periods are properly implemented

At the undergraduate level, the UGC proposes to allow little or no further expansion in enrolment during the Fifth Plan, at least for formal, full-time studies Expansion, if any, will come about only through part-time evening classes or correspondence courses It has also been decided that the affiliated colleges would receive UGC assistance during the Plan, primarily for strengthening their faculty, improving their library and laboratory facilities, and installing workshops and related facilities The colleges have also been advised to give priority to re-orienting their courses so that they become relevant to local, regional and national needs and emphasise utilisation of available natural resources

● The UGC report for 1972-73, analysing the emerging problems and perspectives of higher education in India, blames unrest among students on the present system of examination, obsolescence of university curricula and the lack of good social life in colleges and universities

The report points out that enrolment in university-level courses has left growth rate of the national economy way behind, resulting in lower per capita investment in higher education and also deterioration in standards of institutions where facilities are being stretched to breaking point in accommodating increased student populations (In 1972-73, there were 35 44 lakh students enroled in 4,153 colleges in the country)

Referring to growing unemployment among the educated, especially women and those who graduate from the universities in sciences and various professional courses, the report says that

Educating India

from Narender K Sehgal, Jullundur

much undergraduate education is not relevant either to the abilities and aptitudes of students or to the needs of the nation With a very high failure rate at first degree level (about 50%) and large proportions of students being placed in the "third division" (aggregating less than 50% in an examination) at the postgraduate level, the report feels that "the present system of higher education is generating much waste and stagnation" and that "this raises serious doubts about the well being of the university in India "

The report further points out that the spread of higher education has not been very even, both area-wise and population-wise N e i t h e r backward areas, nor students from these regions, have been able to get their fair share of facilities or opportunities for higher education — meaning that disparities have grown worse

The commission blames on paucity of funds its inability to play a role in developing a system of higher education best suited to the genius of the people and the development of the country It proposes a dialogue with the universities with a view to determining what can be done during the period of the Fifth Plan to make higher education a "little more meaningful and ultimately a fit instrument of change and development "

● The University Grants Commission has proposed 'radical' reforms in the present examination system Meerut University, in Uttar Pradesh, is likely to be the first to try out the reform on an experimental basis during the next academic year Similar experiments are also expected to be launched in universities in other states

Central to the proposed reforms is what is called a 'question bank' Such a bank will have up to 100 questions which will be published and made available to both students and teachers at the beginning of the academic term Seventy-five per cent of the questions asked in an examination will be taken out of the bank and the rest (25%), mostly numerical problems, from outside The curriculum and the question bank for each course of study will be framed by Boards of Studies to be constituted for the purpose

Also proposed is a card system for the examination questions The cards containing questions, to be answered by individual examinees, will be picked up using a random process from a collection (This will hopefully help reduce the use of unfair means at examinations—a very prevalent feature at present) The candidates will be able to appeal to a committee if, on receiving the evaluated answer books, they feel dissatisfied with the evaluation The committee will consist of the college principal, the teacher for the course and a representative of the students

A university will be free to cancel a student's admission if he/she gets a 'D' grade in all his/her examinations and assessments The grades A, B, C and D will replace the 'marks' being awarded in evaluations at present

Universities as well as colleges will be required to conduct sessional or continuous assessments of students which will be shown on their grade sheets separately, as this will be aimed at measuring their "essential abilities" The name of colleges will also be mentioned on the degree/diploma of a qualifying student The performance assessment of students will be required to be made over well distributed intervals of time

Meanwhile the Union Education Minister recently told a meeting of the consultative committee attached to his ministry that the present examination system had collapsed completely and that reforms in this direction were urgently needed He informed the members that 11 out of the 12 universities selected for launching examination reforms suggested by the UGC had already set up special implementation committees for the purpose ☐

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the production ot tufted carpets"

Cooperation in the field of raw materials likewise ranges from window glass and timber products to "the enrichment of uranium in the Soviet Union from the raw material of British customers '

In general, the technological section of the programme tends to become bogged down in detail, and the order of the items would surely form a fascinating study for the psychologist (cars, for example being listed immediately before toys) There is, however, a general overall stress on computer hardware and software, power production, including atomic power stations, heavy ridge group of exposed sediments ☐

Wrong skull

THE skull illustrated on page 578 (February 20) was in fact KNM-ER1470 found at East Rudolf in 1972 It has been assigned to the genus *Homo,* sp indet , and was found during prospecting in area 131 on the Karari industry and means of transport ● ☐

SCIENCE and technology are receiving a new emphasis in the Arab world, partly as a result of the increased wealth of the oil-rich countries The new source of wealth has made possible educational and research facilities, new institutions and programmes hitherto out of reach for most of the countries in the area The first Conference of Ministers of Science and Heads of National Science Policy Organisations of the Arab States was held in Baghdad last year, and an Arab League Educational, Cultural and Scientific Organisation (ALESCO) has been formed in recent years to promote Arab culture, including science and technology A number of Arab states have formed—or are forming—science policy machinery and programmes, emphasising applied research

Egypt has traditionally been the most advanced Arab nation in science, and has built up a large pool of professionals who are much in demand in other Arab states Iraq, which today has an ambitious five-year science plan, takes a distant second place But while Egypt is poor, Iraq is now rich, and any promising research project can obtain funds there Yet, ironically, Iraq is poor in personnel

Trained personnel, in fact, are the main need of the Arab countries generally Countries relatively rich in scientific manpower—Egypt, Lebanon and Jordan—are supplying the rest with personnel Countries like Kuwait and Saudi Arabia are busy building laboratories and universities and advertising openings for staff

Because of the wide differences in resources between the Arab states, Dr Ibrahim R Shimi, head of the chemistry department in Ain-Shams University, Cairo, has proposed that a research council be established in the League of Arab States to which member states should allocate a proportion of their GNPs This council would set up specialised research centres dealing with problems common to the area as a whole, such as cultivation of arid lands and desalination of sea water

The Arab countries' experience with science policy formulation is varied Egypt's has been complex, and began with its Science Council, established in 1956 The Science Council produced a national research plan for the universities, the new National Research Centre laboratories and the specialised institutions belonging to the ministries

This council was superseded in 1961 by the Ministry of Scientific Research In 1965, the Supreme Council of Scientific Research came into existence —but it was short lived and was replaced by the Ministry of Scientific Research in 1968 The Ministry in turn was replaced in 1971 by the Academy

of Scientific Research and Technology The president of the academy, at present, Dr A Abou-El-Axm, has ministerial rank and is responsible to the Prime Minister

The academy not only formulates science policy but supports research and technology through grants It also coordinates major projects, participates in development of science curricula, supports scientific conferences, and organises scientific publications and the popularisation of science, among other things In addition, it is responsible for a number of research institutions in such areas as atomic energy, oceanography, petroleum and metallurgical research, and research on bilharzia

The Arab World science revival

from David Spurgeon

Grain sorghum research, Lebanon

By comparison, Lebanon's history of science policy has been uncomplicated It began in 1962 with the establishment of the National Council for Scientific Research (NCSR), and this single body not only was the first science policy-making body, but has continued as the sole one The situation is further simplified by the fact that the same body that formulates policy also sees that it is carried out

Lebanon went about its science policy planning deliberately and directly The underlying assumption was as stated in its Five-Year-Plan for the Organisation and Development of Scientific Research in Lebanon

"Lebanon is a small country with limited human and material resources Its aspirations, whether for a higher standard of living or an enhanced cultural standing, can therefore only be conceived and implemented through utilisation of its resources, human and material at the maximum possible

efficiency"

The NCSR therefore went about setting up the national science plan by first asking the Ministry of Planning what the country's overall objectives were It promptly received a reply listing maximum regular increase of the national income, equitable distribution of the national income among citizens, full employment, establishment of an equilibrium among the various economic sectors, and rational distribution of the population over the territory of Lebanon

Eighty per cent of the plan deals with oriented research according to priorities carefully and simply laid out, 20 per cent deals with non-oriented or basic research Themes accorded top priority are Lebanese climate, conservation of the natural milieu, health and disease in Lebanon, housing, and what are called "the essentials of productivity"— Lebanese raw materials, soil and water, and marine resources NCSR's 1973 budget was 5,250,000 Lebanese pounds

Some examples of the projects funded by the NCSR illustrate the strongly practical philosophy behind the plan development of a high-protein, vitamin and mineral biscuit for low income groups, the study of Lebanese plants used in folk medicine with the aim of isolating and analysing their active elements with a view to their exploitation or synthesis, the industrial production of fish and molluscs using recycled human wastes, and research on processing of surplus fruit crops

Joseph Naffah, Secretary-General of the NCSR, says that the practical orientation was adopted partly to attract attention to the accomplishments of science and to improve the climate of research, because in Lebanon, "the scientist is not respected in the sense that the self-made man is respected"

There is also a surprising amount of attention being paid to the popularisation of science Salah Gelal, science editor of Cairo's newspaper *Al Ahram*, heads a staff of 12 science reporters and, with the financial backing of his newspaper, organises science clubs and fairs for Arabic youth Last year, in Baghdad, UNESCO and the Union of Arab Broadcasters sponsored a meeting on the popularisation of science, and training symposia have been held and attended by representatives of a number of Arab countries In fact there seems to be a growing realisation that the real costs of the area's long involvement in war include not only lost lives and material waste, but also a retardation of national development The Arab states see a revival of science as essential to the fulfilment of their nation's potential ☐

correspondence

Visa problems

SIR,—In your issue of December 13, Miss Peller gave an account of her experiences in trying to attend the Ninth FEBS Meeting in Budapest last August As Chairman and Secretary-General, respectively, of the Federation of European Biochemical Societies at that time, we naturally regret that Miss Peller was unable to obtain a Hungarian visa in time, although we have been assured by the organisers of the meeting that a visa was in fact available, unknown to her, in Vienna three days before the meeting started

Because there are no diplomatic relations between Hungary and Israel it was anticipated that it would be difficult for biochemists in Israel to apply for Hungarian visas This problem was discussed during a visit to Budapest in February 1974 by the then Secretary-General of FEBS and Professor S G Van den Bergh, the FEBS meetings adviser In consultation with the organisers of the FEBS meeting (the Hungarian Biochemical Society and the Hungarian National Academy of Sciences) a special procedure was worked out which enabled Israeli biochemists to send their passports some months before the meeting by diplomatic mail to London, where the FEBS Secretary-General obtained Hungarian visas on their behalf within 24 hours and then returned the passports and visas to the applicants in Israel, again by diplomatic mail Though somewhat elaborate, these arrangements worked satisfactorily in the case of applications received in good time through the Israeli Biochemical Society They did not, however, cater for biochemists like Miss Peller, who were not resident in Israel at the time and therefore applied directly to Hungarian consulates

In Miss Peller's case there is at present a fundamental discrepancy between the information she received at the Hungarian consulate in Vienna where she was told she was on a "black list" and would not be admitted into Hungary and telegrams she received by one of us (H R V A) from the meeting organisers some days before the meeting started stating that her visa had been granted Although we have not yet been able to resolve these apparent contradictions, we believe that a genuine effort was made both by the organisers of the FEBS meeting and the authorities in Hungary to establish a satisfactory procedure for dealing with all visa applications and there is no evidence to suggest discrimination in the issue of visas

Nevertheless, we think that there are several important lessons to be learnt by the organisers of future international conferences, as well as by intending participants Principally, there is a need to ascertain at an early stage of planning a meeting the precise official regulations of the host country concerning visa applications, to include this information in the circulars sent to all intending participants, to make and publicise special arrangements for citizens of countries which do not have normal diplomatic relations with the host country, and to urge participants to apply early for visas Finally, we believe it would be useful, particularly in the case of large conferences, to appoint a member of the organising committee to be responsible for dealing with urgent visa problems and to announce his name, address and telephone number well in advance of the meeting so that contact can be made quickly if and when participants have special difficulties

Yours faithfully,
L L M van Deenen
University of Utrecht
H R V Arnstein
King's College, London

SIR,—In continuation and support of S Peller's letter (December 13) I would like to point out that I was, obviously on the same grounds, subjected to an identical procedure by the diplomatic authorities of the host land of the Extraordinary General Assembly of the International Astronomical Union at Torun in September 1973—in spite of possessing an official invitation

Although I was far from active politically or in any other way that could interfere with the interests of the host country, and despite rigorous adherence to the instructions printed on the invitation, I was refused an entry visa and thus prevented from attending the assembly I wish to thank all colleagues who tried—in vain—to change the decision at the very last moment

Yours faithfully,
Andrei R Serban
Heidelberg

IQ Statistics

SIR,—A recent editorial (September 27) has drawn attention to the unfortunate social consequences of the recent controversy about the possibility of genetic contributions to the observed differences between the average IQs of different races It does not seem to have been noticed that many of the contributors to the debate, on whatever side, have quite misunderstood the logic of the relevant statistical methods

If, as a first approximation, we assume that the observed IQ of an individual is the sum of a genetic component, G, and an environmental component E, so that $I=G+E$, it has become customary to define a 'coefficient of heritability', h^2, as the ratio of the variance of G to that of I In doing this the tacit assumption is made that G and E are uncorrelated in the population so that the variance of I is the sum of the variances of G and E This is usually the case for quantitative characters in animals and plants For human IQ, however, there is no doubt whatever that G and E are correlated, environment (both physical and psychological) being strongly associated with intelligence Thus there is no point in attempting to estimate h^2 since it cannot be defined, let alone estimated, and attempts to estimate in are a misapplication of the analysis of variance

It is of course, true that it might be possible in theory (but very difficult in practice) to divide the variance of I into three terms, the variances of G and E, and their covariance, and even to go further and split the variance of G into linear, dominance and epistatic components

Even if this could be done, however it is clear that such components of variance within a population throw hardly any light on the differences between populations This is easy to illustrate on animal and plant populations where h^2 within populations, when it can be defined, has no relationship to population differences

At present there exist no methods of estimating differences in mean levels of G, and there is no evidence of any kind which suggests that negro intelligence is on the average, less than, equal to or greater than that of white people These three hypotheses are not intrinsically unplausible but those who think they can be tested empirically have been inadequately instructed in the analysis of variance by their statistical colleagues

Yours faithfully,
P A P .Moran
The Australian National University

University competition

SIR,—J B S Price's lament about unfair competition from university-inspired science-based companies cannot be allowed to go unanswered

One has sympathy for a company like Mr Price's which attempts to make profit from difficult technology—too often the development costs are unlikely to be justified by the potential market Science often requires such development to advance a subject, however, and without it no market could possibly develop It is just such devices that are likely to be spawned by university staff The important question for the scientific instrument industry in the UK is How do we get such schemes financed? Certainly not from companies like Mr Price's, in 1975 And only partially from the NRDC—50% on very hard terms

I am surprised he fears university competition on hardware No university can operate such activities except on a very small scale and has no sales organisation If the scale is small, it is unlikely to be profitable for such companies as Grubb Parsons in any event and perhaps should be tackled by a smaller company

He has less to fear than he thinks, such operations are rarely very professional and most buyers won't trust universities to come up with the goods —again keeping the scale small There are, of course, university entrepreneurs who expand their activities rapidly, becoming fully commercial and professional They then, however, face all the problems of production facilities, cash-flow and overheads that Mr Price does Their sole advantage is their enterprise and enthusiasm

I write because a negative attitude to this problem will only lead to featherbedding, with enough of that, the competitiveness of British scientific industry will continue to diminish to the disadvantage of our balance of payments

Whatever is said, we must find an effective way to turn the country's considerable investment in university research into industrial progress

Yours faithfully,
S D SMITH
Heriot-Watt University

Taboo research?

SIR,—Some 20 years ago the first infectious virus was reconstituted in the laboratory from component chemicals, and some religious circles, Catholic as well as fundamentalist, were wondering whether to disapprove such research activities on the grounds that they were approaching too close to God's territory They wisely and fortunately remained silent

This week an international group of scientists and science administrators

met at a resort on the coast of California to discuss the merits, or rather dangers, of a much more advanced research on similar lines which is going on all over the world I hope that this group will show as much wisdom as the churches did earlier Taboos to block, or guidelines to channel man's curiosity have never succeeded in the long run, and usually they appeared ridiculous a few decades later They are as old as the caveman's wife telling her mate not to bring that burning piece of wood from the tree hit by lightning into the cave, and not to pick up that club-shaped branch when attacked by a bear Yes, there was danger in these activities—man could and did hurt himself as a consequence of each of these acts The end effect of these activities, however, is that *homo sapiens* has greatly multiplied and that ever more members of the species live longer, more healthily and more comfortably than before

Another result is that people are now able to arrange world-wide meetings, with busy scientists travelling through the air, very rapidly and rather safely, to discuss further steps in unknown directions, and their dangers, and guidelines to hamper these steps Man also developed remarkable means of communication so that the original fears and misgivings of the caveman's mate ('caveperson', so as not to prejudge who brought the fire in) are now brought to everybody's attention and the weak, timid, conservative (not to say conservationist) members of the species may through their numbers be able to exert real pressure on the Promethean creative curious individuals, to make them cease and desist The human species as a whole has, however, survived, and benefited, from the discovery and utilisation of fire, the wheel, powder, the steam engine, vaccination, electricity, dynamite, fluoridation and atomic energy It will surely hardly notice that some scientists are suffering from extrapolatotis and super-scrupulosis Thus the danger of the present meeting to future research is surely small, if any, and of short duration The fact that this is a court of scientists sitting in judgment on scientific research makes it more disturbing and regrettable, however, in that it shows to what extent the anti-intellectual disease of doubting the value of progress and advance of knowledge has spread to the ranks of the presumed Prometheans

H FRAENKEL-CONRAT
University of California

Naming names

SIR,—I would like to align myself with those anthropologists, such as Robin A Drews, who have a proprietary attitude

toward the proper spelling of Peking man (Peking, please, not Pekin) My own proprietary attitude extends to the Peking duck (*Anas platyrhynchos*) Although it is probably too late to do anything about Pekin, Illinois, USA, I do hope we can rescue the " big, white duck known as a Pekin variety" from nomenclatural ignominy

First of all, there is no Pekin, China The name Peking comes from two Chinese characters, the first of which (pei) means northern and the second of which (ching or king) means capital Although some anthropologists suspect 'Americanism at work' in the base misspelling of their favourite fossil remains and my favourite domestic waterfowl, I have developed a different hypothesis in the course of years of grappling with this persistent problem in orthography

The Peking duck originated in China and was first noted in the USA around 1870, but I can't determine from whence it came I had always assumed Europe While reading some marginalia in Jean Delacour's engaging volumes on waterfowl systematics, I observed that he claimed to have visited Pékin, China in the course of his studies I was a little dismayed that such an obviously literate and well travelled scientist could be found among the ranks of orthographic incompetents until I learned that in the French language Peking (whether city, duck, or man) is Pékin Thus, I arrived at an essentially francophilian explanation of the entire business, to wit, the forlornly foreshortened Pekin represents the uncritical (and incomplete) adoption of the French word for Peking *Voilà!*

GILBERT GOTTLEIB
Raleigh, North Carolina 27611

SIR,—The term 'exobiology' excludes life on Earth It is, of course, impossible at present to study exobiology without reference to life on Earth Even if several extraterrestrial life forms were discovered tomorrow, nobody would study them without comparing them with terrestrial life Thus there is a need for a word which includes all life forms in the universe

The term cosmochemistry is already well established and has been defined as 'the branch of science which treats the chemical composition of the universe and its origin and evaluation" It would thus seem to be appropriate to use the term cosmobiology for the study of life in the Universe I believe that such matters of terminology are not trivial, for the manner in which a science is divided into branches has a significant effect on the development of that science

Yours faithfully,
A A COTTEY
University of East Anglia

news and views

Chloroplast protein synthesis

from Harry Smith
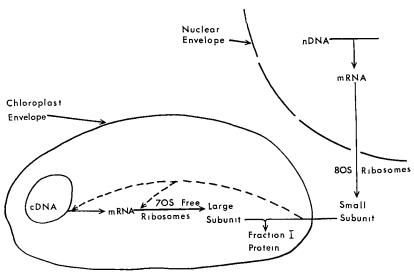
READERS of the more distinguished biochemistry and molecular biology journals could be forgiven for concluding that plants do not have genes, nucleic acids, proteins, enzymes and intriguing regulatory processes such as are possessed by respectable organisms like *E coli* and HeLa The omissions are doubtless due to the small amount of work in progress in these areas of plant science, which is, in turn, largely caused by lack of interest in, and knowledge of, plants on the part of traditionally trained biochemists It is, therefore, refreshing to see a group tackling one of the central problems of plant molecular biology and in the process producing a concept of interorganelle regulation which may have important implications for other organisms

R J Ellis and his colleagues at the University of Warwick have for several years now been studying the complex problem of the synthesis of Fraction 1 protein in leaves This protein, which may represent up to 50% of the total soluble leaf protein, is only found in its intact state within the chloroplasts, where it carries the catalytic function of ribulose-1,5-diphosphate carboxylase, the enzyme responsible for photosynthetic carbon fixation The molecule has a molecular weight of about 525,000 and probably consists of eight identical large subunits (molecular weight about 55,000) and eight to ten identical small subunits (molecular weight about 15,000) Genetic experi-

ments have shown that the functional gene for the large subunit is present in the chloroplast genome, whereas that for the small subunit is in the nuclear genome Moreover, earlier inhibitor experiments indicated that the large subunit is synthesised only on chloroplast ribosomes *in vivo*, and the small subunit on cytoplasmic ribosomes There is thus a fascinating problem of interorganelle communication and integration.

In 1973 Blair and Ellis (*Biochim Biophys Acta*, **319**, 223–234) showed that intact, isolated pea chloroplasts when driven by light energy, incorporated labelled amino acids into the large subunit of Fraction 1, the only soluble polypeptide to be synthesised Its identity was established by comparing a tryptic map of the labelled peptides with that from authentic Fraction 1 large subunit Subsequently, Eaglesham and Ellis (*Biochim Biophys Acta,* **335**, 396–407, 1974) showed that isolated chloroplasts in the light incorporate label into six separate membrane-bound products which were considered to be membrane proteins Other workers have obtained similar results using spinach chloroplasts, but in this case upwards of four soluble proteins, and nine membrane proteins, became labelled (Bottomley, Spencer and Whitfield, *Archs Biochem Biophys ,* **164**, 106–117, 1974) The concept has thus developed that at least Fraction 1 protein large subunit and a

number of chloroplast membrane proteins are synthesised on chloroplast ribosomes The location of the genes for the membrane proteins is not yet known

Hartley, Wheeler and Ellis have now demonstrated (*J molec Biol ,* **91**, 67–77, 1975) the existence of the messenger RNA for the large subunit by the only real criterion—its *in vitro* translation into a recognisable polypeptide Using a cell-free *E coli* preparation as a heterologous protein-synthesising system, they showed that the Fraction 1 large subunit is synthesised when total spinach chloroplast RNA is presented as the message Two major labelled products were found, both in the cell-free heterologous system, and as a result of protein synthesis by intact light-driven chloroplasts One of these, with a molecular weight on SDS gels of 52,000, had a closely similar chymotryptic map to that of authentic Fraction 1 large subunit The evidence for the identity of the labelled product with Fraction 1 large subunit seems most convincing

Since the large subunit is coded for in the chloroplast genome, its messenger RNA is present in chloroplast RNA, and it is synthesised on chloroplast ribosomes, some control of synthesis must be exerted such that the relative proportions of large and small subunits are coordinated In the January issue of *Phytochemistry* (**14**, 89–93, 1975) Ellis puts forward a very intriguing hypothesis for the mechanism of this integration He shows that MDMP (2(-4-methyl-2,6-dinitroanilino)-N-methylpropionamide), a highly specific inhibitor of initiation on 80S ribosomes, inhibits the synthesis of both the large and the small subunit when applied to intact pea leaves MDMP does not inhibit protein synthesis in chloroplasts On the basis of this evidence, a model is proposed in which the small subunit is thought of as a positive factor required for the initiation of either the transcription of the messenger RNA for the large subunit, or for its translocation (see figure) On this model, the synthesis of the large subunit by isolated chloroplasts represents run-off of preformed polyribosomes, which accounts for the observed rapid falling off in the rate of protein synthesis This elegant and painstaking work convincingly demonstrates that plant molecular biology is not dead, but is merely awaiting the attention of competent and dedicated biochemists

Cause and effects of global cooling

by John Gribbin

A RECENT flurry of papers has provided further evidence for the belief that the Earth is cooling There now seems little doubt that changes over the past few years are more than a minor statistical fluctuation

Last year, Kukla and Kukla (*Science,* **183,** 709, 1974) reported satellite observations of the sudden growth of ice cover in the northern hemisphere in the winter of 1971–72, and suggested that the increase was equivalent to one-sixth of the change needed to bring about a full ice age On page 45 of this issue of *Nature,* Wahl and Bryson compare recent sea surface temperature patterns with those of cooler regimes in the past, and conclude that over the period from 1951 to 1972 there was a decline corresponding "to a return of about one-sixth of the way to a full ice age" It is tempting to suggest that the change in sea surface temperatures contributed in some way to the spread of ice noted by Kukla and Kukla

But there is as yet no evidence that further cooling is likely in the immediate future The observed cooling corresponds to a re-establishment of the 'Little Ice Age' which persisted for several hundred years up to the end of the nineteenth century, it may be that all that has happened since 1950 is that the unusually mild spell of the first part of this century has ended Over the past millenium, icier conditions than those of today have been the norm Indeed, ice ages have been common over the past two million years But on longer time scales still, such cool phases are relatively rare events—and there is now a plausible model to explain why this should have been so

At present, the land surface of the Earth is concentrated in the northern hemisphere, around the almost land-locked polar sea This is a situation which can encourage the spread of ice much more than if warm water could easily penetrate to the polar sea, although of course polar ice forms even more easily when the pole is covered by land, as in Antarctica today It seems that over the past couple of million years this distribution of the continents has resulted in a fairly delicate balance between ice ages and inter-glacial regimes If ice cover is established, its high albedo is enough to maintain glacial conditions for a long time, but once the ice has gone a definite trigger (such as volcanic activity or changes in the Earth's orbit, see page 20) is needed to re-establish it

Calculations presented by Sellers and Meadows (on page 44 of this issue) support the commonly held view that the present-day distribution of northern continents may be the root cause of the wave of ice ages in the past two million years Neglecting other effects, if the continents were gathered in a belt around the equator, Sellers and Meadows find that the difference in albedo from conditions like those of the present would raise the global mean surface temperature by 12 °C

Much more work of this kind needs to be done before plate tectonics can provide a full insight into the long-term changes in patterns of glaciation, but at least the link between continental drift and ice ages is now beginning to be put on a quantitative basis

Eukaryotic operon

from P J Ford

GENETIC regulatory systems in prokaryotes are quite well understood compared with those in eukaryotes Regulator genes code for regulator substances (usually proteins in prokaryotes) which function by interacting with regulator sites (operators) in the DNA adjacent to the structural genes under control and with small molecules (either inducers which are usually substrates or repressors which are usually end products) which alter their affinity for the regulator sites A simple model supposes that regulator substances exist in two forms, one capable and one incapable of binding to the regulator site Inducers and repressors shift the equilibrium between these two forms

Four types of gene control circuit are theoretically possible using these components Negative control exists when the binding of regulator protein (repressor) at the regulator site inhibits gene transcription In this case gene activity may be restored (induced) by inactivation of the repressor in the presence of inducer or eliminated (repressed) by activation of the repressor protein in the presence of co-repressor substance which is essential for repressor activity Positive control exists when binding of regulator protein (inducer protein) at the regulator site is essential for gene transcription Inducible positive control is found when the presence of an effector substance (co-inducer), as well as inducer protein, is required for gene activity and repressible positive control occurs when the co-repressor inactivates the inducer protein, eliminating gene activity

Regulator gene mutants in negative control circuits must be recessive if structural gene function occurs in the absence of inducer (constitutive) or in the presence of co-repressor (de-repressed), or dominant if there is no expression in the presence of inducer (uninducible) or in the absence of repressor (super-repressed) In positive control circuits the dominance and recessive relationships are reversed Mutations in regulator sites are always dominant when linked (*cis* configuration) to structural genes and recessive when unlinked (*trans* configuration) Mutations in regulator sites of negative control circuits are constitutive or de-repressed and of positive control circuits are uninducible or super-repressed

These four theoretically possible types of simple control circuit are not equally used Most control circuits in prokaryotes are under negative control and either inducible, like the lactose operon, or repressible, like the tryptophan operon An inducible positive control system is the cyclic AMP system which controls several inducible operons (galactose for instance) and such operons are under dual control In this system cyclic AMP, which is neither a substrate for, nor a product of the enzyme pathway, activates a binding protein which is required for transcription of the operon A further complication may arise when a regulator protein acts both in a negative and a positive way such as is found in the control of the arabinose operon In this circuit the regulator protein acts as a repressor in the absence of arabinose but in its presence binds to an initiator site, allowing gene activity

A second type of control site (called promoter or polymerase binding site) which may be completely separate from or partially overlap the operator site has been found in many prokaryotic control circuits Promoter sites are usually located distal to negative and proximal to positive control operators Theoretically, positive control may be exercised by interaction of control proteins (sigma factors) with the polymerase core enzyme altering its specificity towards promoter sequences Mutations in promoter sequences will

US Department of the Interior

PART of North America's largest herd of caribou which migrates yearly between forest and tundra in northwestern Alaska. They regularly cross the basin of the Noatak river, one of the proposed National Wildlife Refuges which will be created as a result of the Alaska Native Claims Settlement Act of 1971.

The Noatak region lies in a transition zone between northern boreal forest and arctic tundra. With 500 species of vascular plants the flora of the proposed 7.5 million acre refuge is as great as that of the whole of Greenland or Iceland. The Department of the Interior intends to establish a 20-year moratorium on any developmental activities in the region to allow a comprehensive analysis of this intact slice of arctic ecosystem to be conducted. At a time when immense pressures are being applied to arctic ecosystems, in the form of roads, pipeline corridors and oil and gas extraction fields, the decision is as wise as it is opportune. What is learned from the analyses will benefit not only the future of the Noatak region but perhaps the future of all arctic lands.

be *cis*-dominant and *trans*-recessive and may be either 'up' (increasing transcription) or 'down' (decreasing transcription) in phenotype.

Clustering under common genetic control of the structural genes for enzymes of the same metabolic pathway is seen in prokaryotes but not eukaryotes, and represents an apparent distinction between these major phylogenetic groups. Taken together with their greater genomic complexity, the presence of large amounts of redundant DNA sequence and their intricate patterns of development and differentiation one might expect the gene control processes of eukaryotes to involve something more than those seen in prokaryotes. But the genetic analysis of gene control in eukaryotes has hardly begun and it is too early to say whether this expectation will be fulfilled. Two articles (Arst and MacDonald; page 26; Arst and Scazzochio, page 31) in this issue of *Nature* identify regulator sites involved in the control of two enzyme systems in the ascomycete fungus *Aspergillus nidulans*.

Proline catabolism in *Aspergillus* involves three enzyme activities: a permease, an oxidase and a dehydrogenase, which are under three distinct forms of control—induction by proline, nitrogen metabolite repression, and carbon catabolite repression. A probable structural gene for the permease (*prn*B) is tightly linked to a probable structural gene for a component common to both the oxidase and dehydrogenase (*prn*A). *prn*A⁻ mutants lack oxidase and dehydrogenase and *prn*B⁻ mutants lack the permease. All three activities are inducible, permease and oxidase are carbon catabolite repressible (dehydrogenase was not investigated), but only oxidase and dehydrogenase are nitrogen metabolite repressible—the permease is not. The two repression systems seem to act independently of each other. A gene *are*A is probably a regulator gene producing a positive control element which allows the synthesis of a large number of enzymes and permeases involved in nitrogen metabolism. One class of mutants (*are*Aʳ) leads to inability to use a variety of nitrogen sources including proline and presumably lacks a functional *are*A product. Second site mutations, called *prn*ᵈ, restore the ability of *are*Aʳ mutants to utilise proline as a nitrogen source. *prn*ᵈ mutations are *cis*-dominant, *trans*-recessive mapping betwen but not suppressing or allelic to *prn*A⁻ or *prn*B⁻ mutations, which are themselves tightly linked. *prn*ᵈ mutations do not significantly affect the induced levels of enzymes and are probably best interpreted as carbon catabolite derepressed since uninduced levels of the products of both *prn*B (which is not ammonium repressible) and *prn*A are higher in *prn*ᵈ mutants than in wild type in the presence of partial glucose repression.

The model favoured by Arst and MacDonald to explain their results of a regulator site (*prn*ᵈ) located between two structural genes (*prn*A and *prn*B) is reminiscent of the divergent arginine operon in *E. coli*. They suggest that *prn*ᵈ may be the binding site for a negative control element produced by a gene, such as *cre*A, identified by mutations which are commonly recessive and lead to carbon catabolite derepression.

In a second paper Arst and Scazzochio describe the isolation of a second site revertant (*uap*-100) of a mutant *are*-102 which has reduced levels of, among other enzymes, uric acid/xanthine permease. *uap*-100 is tightly linked to the probable structural gene (*uap*A) for uric acid permease. It is *cis*-dominant, *trans*-recessive and after maximal induction has a permease activity 2.5 times that of fully induced wild type. They interpret these results as identifying a regulator site with promoter-like properties.

Although the description of these two control systems is at an early stage it seems likely that Arst and his colleagues have identified an operon-type structure involved in proline catabolism and a promoter-like regulator site adjacent to the putative structural gene for uric acid permease in the eukaryote *Aspergillus nidulans*. The recent results of Hynes (*Nature*, **253**, 211; 1975) suggest that a similar regulatory mechanism affects the structural gene for acetamidase in *Aspergillus nidulans*.

Are mantle plumes jets or blobs?

from Peter J. Smith

IN a series of reports appearing over the past two years or so, Schilling and his colleagues (see, for example, Schilling, *Nature*, **242**, 565; 1973 and Hart *et al.*, *Nature phys. Sci.*, **246**, 104; 1973) have presented geochemical evidence which they claim is consistent with, and thus appears to support, the existence of a rising mantle plume beneath Iceland. In particular, they have interpreted the chemistry of basalt lavas from Iceland and surrounding areas in terms of two distinct magma sources—the conventional asthenosphere and a plume of hot primordial material derived from a much greater depth in the mantle. O'Hara (*Nature*, **243**, 507;

1973), on the other hand, criticised this view on the grounds that the data could as easily be explained in more conventional terms. He pointed out that the geochemical variations radiating from Iceland could be caused not by the mixing of two primary magmas but by differential fractional crystallisation of material from a single source; and he now (in *Nature*, **253**, 708; 1975) reiterates, clarifies and expands the arguments in support of his case.

In his original article O'Hara seemed to imply that a choice would have to be made between two mutually exclusive models. He now suggests that fractional crystallisation and a mantle plume might be compatible under certain circumstances but that, if they are, Schilling's particular interpretation involving relatively undepleted plume material must certainly be ruled out on grounds of density alone. Schilling, however, continues to stick to his own model involving a plume "made of slightly lighter and more primordial material"; and he and Noe-Nygaard (*Earth planet. Sci. Lett.*, **24**, 1; 1974) have now extended the investigation to cover variation in the behaviour of the Icelandic plume with time.

In the light of Schilling's view that the plume comprises material chemically and isotopically distinct from that of the asthenosphere (which is penetrated by the plume from below), Schilling and Noe-Nygaard envisage three different types of plume–ridge relationship. Their 'normal ridge segments' (NRS) remote from any plume have normal ridge elevation, well developed and symmetrical magnetic anomalies and typical oceanic crustal thickness and structure. The material rising to form new lithosphere (in response to spreading rather than as a cause of it) is derived from the asthenosphere, and the resulting basalts are tholeiitic, depleted in large ionic lithophile elements and low in radiogenic isotopes, and have $(La/Sm)_{EF}$ ratios <1.

The 'plume ridge segments' (PRS) directly over plumes have higher elevation, branching aseismic ridges or island chains, crustal thickness and structure intermediate between the typically oceanic and typically continental, and higher geothermal gradients. Here plume material is forced into the gap left by diverging plates, usually in quantities sufficient to more than fill the gap and thus allow the accumulation of plateau basalts and the formation of anomalously thick oceanic crust. In this situation new lithosphere over a PRS will be derived entirely from the plume, will have $(La/Sm)_{EF}$ >1 and will have radiogenic isotopes characteristic of the particular plume. But when and if the plume flux decreases to below the quantity required by the diverging plates, asthenospheric

material will mix with the plume material leading to hybrid lavas with intermediate $(La/Sm)_{EF}$ values and radiogenic imprinting. Finally, there are also 'transitional ridge segments' (TRS) between NRS and PRS, but for the time being these are ignored because of their complexity.

With this model (NRS and PRS only) in mind, Schilling and Noe-Nygaard have investigated the rare earth element (REE) chemistry of the 3,000 m thick lava sequence in the Faroe Islands. This pile of plateau lavas contains two clear marker horizons which define a series of aphyric quartz tholeiites (lower), a series of porphyritic quartz tholeiites (middles) and a series of olivine tholeiites (upper). The geochemical study shows that, with the exception of one flow in the middle series, all the flows of the lower and middle series have light REE enrichment with $(La/Sm)_{EF}>1$. By contrast, the flows of the upper series fluctuate rapidly between REE depletion with $(La/Sm)_{EF}<1$ and REE enrichment, ending (at the top) with flows predominantly depleted. Schilling and Noe-Nygaard claim, giving reasons, that this two-component pattern cannot be attributable to differing fractional crystallisation or partial melting. They argue instead that the data support their two-source model. The lower and middle series are plume-derived, with the exception of the anomalous middle series flow which marks the first appearance of asthenospheric material. In the upper series there are rapid

changes between flows mostly plume-derived and flows mostly asthenosphere-derived, with the most recent activity being mainly of the latter type.

But why did the change from plume material to largely asthenospheric material take place? There are two possibilities within the scope of the Schilling–Noe-Nygaard model; either the plume must have decreased in intensity or the spreading rate at the ridge must have increased, thereby allowing the asthenosphere to acquire greater influence. According to Tarling and Gale (*Nature*, **218**, 1043; 1968) the ages of the Faroes basalts lie between 60 and 50 million years, and according to Vogt and Avery (*J. geophys. Res.*, **79**, 363; 1974) spreading in the area was decreasing rapidly at that time. The intensity of the plume must therefore also have decreased rapidly. Before it did, however, spreading rate and the flow REE chemistry were comparable to those relating to Iceland during the past few million years, which suggests that the plume intensities then and now were similar. This, in turn, would imply that the plume intensity went through a minimum some time between 50 and 10 million years ago.

Insofar as so very little is known about the origin of supposed mantle plumes and the conditions of their existence, there is presumably no basis for ruling out such temporal variations in plume intensity. On the other hand, in view of the large inertia of thermal systems of the required size, it may be difficult to understand how a plume envisaged as a continuous jet of material could vary in intensity quite so rapidly as the geochemical properties of the Faroes basalts imply. One way in which the REE data and a continuous plume could be reconciled would be to postulate sudden rift jumping and tapping of asthenospheric material at the plume margin, but there is apparently no observational support for this. Schilling and Noe-Nygaard therefore propose that mantle plumes may not be continuous jets at all but may arise as a series of discrete blobs or diapirs. This makes rapid flux variations in space and time much easier to visualise, especially if the rising blobs were to taper downwards rather as inverted liquid droplets.

Reducing exposure of tissues to contraceptives

from our Steroid Biochemistry Correspondent

SINCE the late 1950s when steroids were first used as oral contraceptives many variations in the nature of the steroid

employed, the mode of administration and the dose administered have been tried in order to increase the acceptability of hormonal contraceptives and to eliminate possible side-effects But the fact that the steroids enter the general circulation means that tissues other than those concerned with the control of fertility are exposed to the biologically active compounds, and often for long periods of time In order to overcome these disadvantages the possibility of obtaining an antifertility effect by a more direct action of the steroid on tissues of the reproductive tract, with a consequent reduction in the systemic effect, has been investigated

One interesting development uses the advantageous features of an intra-uterine device and low-dose progestogen contraception (Pharriss *et al*, *Fertil Steril*, **25**, 915–921, 1974) This intra-uterine device is made of an ethylene copolymer in the shape of a T, the stem of which is hollow and contains a suspension of microcrystals of pro-gesterone The rate of release of pro-gesterone from the device is controlled by the ethylene copolymer membrane Initial trials suggested that a pro-gesterone release rate of 65 μg per day was sufficient to provide a contraceptive effect This amount of progesterone is equivalent to only a small proportion of the daily production rate during the luteal phase of the menstrual cycle The present report gives details of the use of this device by more than 3,000 women for more than 25,000 women-months of treatment The efficacy and use effectiveness of the device compared favourably with that of other methods of contraception The preg-nancy rate was only 1% compared with a rate of 18% for a similar T-shaped device not containing progesterone A T-shaped device was used because of its acceptability, easy insertion and re-moval and low natural expulsion rate

This method of contraception has other advantages the active compound is delivered directly to the target tissue and the low release rate of the natural ovarian hormone is unlikely to produce any systemic effect even if any enters the general circulation The sloughing of the endometrium at menstruation prevents an accummulation of the steroid in this tissue In investigations with monkeys using devices containing isotopically labelled progesterone it was shown that some radioactivity did enter the circulation but since the uterus is known to metabolise progesterone it seems that this radioactivity is more probably associated with biologically inactive metabolites than with pro-gesterone itself The total plasma radio-activity remained constant over a one-year period showing that a relatively constant release rate of pro-

gesterone from the device was obtained As with other intrauterine devices this system has the advantage that it does not require any active involvement by women using it

An alternative approach to the problem of reducing the continuous exposure of the tissues to the contra-ceptive steroid involves the administra-tion of a synthetic progestogen at the middle of the menstrual cycle before endogenous progesterone secretion com-mences (Sakiz *et al*, *Contraception*, **10**, 467–474, 1974) In this way the synthetic steroid blocks the pro-gesterone receptors in the uterus and prevents endogenous progesterone from inducing those changes in the uterus which are necessary for implantation to occur In a trial involving 140 women who completed 1,362 treatment cycles, 50 mg of the progestogen ethylnorges-trienone was administered on days 15, 16 and 17 of the menstrual cycle The method proved to be an acceptable form of contraception for most women and many of the minor side-effects seen with other hormonal contraceptives seemed not to occur But there was a failure rate of 9 6 per hundred women years which is too high to be accept-able, about half of these pregnancies were considered to be due to method failure One difficulty of the method is determining the time of ovulation pre-cisely since ideally the progestogen should be administered immediately after ovulation The approach seems however to be a promising one

IgE and immunity to schistosomiasis

from F E G Cox

ONE of the characteristics of the immune response to most infections caused by parasitic worms is the pro-duction of large amounts of IgE It has been speculated that this immuno-globulin (which is associated with various allergies but does not seem to have any beneficial role) originally evolved as an integral part of the immune response to helminths Until recently there has been little real evi-dence to support this hypothesis and theories of immunity to helminths have relegated IgE to a subsidiary role All observers agree that the mechan-isms by which animals rid themselves of worms and resist further challenge are complex but attempts to tie all the available experimental evidence to-gether in a single coherent story make the mechanisms very difficult to com-prehend It is tempting to use all the known facts and to postulate mechan-isms involving cell-bound IgE, serum immunoglobulins, lymphocytes, mye-

loid cells, macrophages, complement and various pharmacologically active substances to account for the immune response

Much of what we know about helminth immunity is based on experi-ments with various nematodes, parti-cularly *Nippostrongylus brasiliensis* in the rat The immune response seems to proceed in three steps damage by anti-body, a cell-mediated response and, finally, myeloid cell degranulation and the production of 5-hydroxytryptamine and histamine which leads directly to the expulsion of the worms The final stage is the one that involves IgE and thus homocytotropic antibody is probably not directly concerned with damage to the worms but plays a peri-pheral part in the development of immediate hypersensitivity Apparent confirmation of this peripheral role comes from the work of Jarrett and Bazin (*Nature*, **251**, 613, 1974) who used the recently available purified rat IgE from myelomas as a reference against which to measure total IgE concentrations in animals infected with *N brasiliensis* They concluded that the bulk of the IgE is surplus to the concentrations expected against known antigens and probably results from potentiated responses to miscellaneous unknown antigens to which the animals had previously become sensitised

In schistosomiasis it now seems that IgE has a central role in the immune response All investigators agree that the stage in the life cycle most sus-ceptible to the immune response is the schistosomula, the young form that penetrates the skin and migrates to the blood vessels in which the adult develops Clegg and Smithers (*Int J Parasitol*, **2**, 79, 1972) demonstrated convincingly that the schistosomula of *Schistosoma mansoni* were killed *in vitro* in the presence of IgG and complement components from immune rhesus monkeys and Capron *et al* (*Int J Parasitol*, **4**, 613, 1974) have found similar cytotoxic activity in humans Antibody-mediated damage can there-fore be regarded as the first step in the immune response to schistosomiasis as in *N brasiliensis* infections

Various kinds of cell have been shown to be involved in immunity to schistosomiasis and it is difficult to determine the actual role of each type Dean, Wistar and Murrell (*Am J trop Med Hyg*, **23**, 420, 1974) have found that rat neutrophils damage schisto-somula *in vitro* by direct contact and by enzymic activity IgG is involved in the attachment of the cells to the worms and complement also seems to play a part in this process although its role is not proven Complement-independent killing of schistosomula by mixed "polymorphonuclear and mono-nuclear" leukocytes has been achieved

by Butterworth, Sturrock, Houba and Rees (*Nature*, **252**, 503, 1974) These authors found that schistosomula were damaged in the presence of normal leukocytes and sera from immune patients The nature of the cells and antibodies involved is not disclosed but it is suggested that cytotoxicity is associated with eosinophils

So far neutrophils and eosinophils have been suggested as effector cells in the immune response but the most recent work in this field also implicates macrophages Capron, Bazin, Dessaint and Capron (*Nature*, **253**, 474, 1974) found that normal rat macrophages adhered to schistosomula in the presence of immune sera *in vitro* Absorption of the immune sera with various anti-rat-immunoglobulin sera showed the immunoglobulin involved to be IgE Further experiments showed that the IgE was specific to the parasite, that macrophage adherence played a part in the killing of schistosomula and that complement was not required for the killing Killing requires the presence of specific IgE which binds normal macrophages to the surface of the worm and, although it may contribute directly to the death of the worm, the main importance of this binding is that it is instrumental in enhancing the lethal effects of immune IgG These experiments draw attention to the possibility of IgE-mediated macrophage adherence in other situations and at last provide a central rather than a peripheral role for IgE in immunity to helminth infections Perhaps we will soon know what IgE really does other than cause asthma and hay fever

Peptidoglycan and the immune response

from a Correspondent

THE bacterial envelope is comprised of the cytoplasmic membrane and the outer cell wall The classic division of bacteria on the basis of the Gram stain turns out to correspond exactly to variations in the chemistry and structural organisation of the outer part of the envelope But in spite of the variations there is a unique component, the peptidoglycan, present in greater or lesser amount in the cell wall of all bacteria The peptidoglycan matrix is built up from strands of chitin-like polysaccharides but with every alternate sugar residue being N-acetylmuramic acid (the 3-O-D-lactic acid ether of N-acetylglucosamine) The acid residues are substituted by amide linkages with short peptide side chains and many of these chains are joined to link adjacent polysaccharide strands together in a meshwork In most—per

haps all—gram negative bacteria and in several gram positive bacilli the peptidoglycan structures differ chemically only in detail although it seems probable that their three dimensional arrangements vary considerably Outside this range of organisms the chemical differences, particularly in peptide structure, multiply enormously

All this has been known for several years It is therefore surprising that so little is known about the effects of the ubiquitous peptidoglycan on mammalian cells Most people are in contact with bacterial peptidoglycan from very early life Certainly peptidoglycan is immunogenic and there must be a continuous stimulation of the immune response by bacteria indigenous to the respiratory and gastrointestinal tracts

And peptidoglycan is of course a component of complete Freund's adjuvant—the suspension of tubercle bacilli which, injected as a water-in-air emulsion with antigen, greatly enhances the immune response to many antigens Does peptidoglycan play any part in adjuvanticity? It now seems clear that it does

Purified peptidoglycan enhances antibody production and induces delayed-type hypersensitivity at rather lower concentrations than those of the whole bacilli used in Freund's adjuvant There is a fascinating argument going on between two French laboratories about the minimum structural features of peptidoglycan necessary for the adjuvant effect Lederer and his colleagues Adam, Ciorboru Ellouz and Petit (*Biochem biophys Res Commun*, **59**, 1317–1325, 1974) have taken degradation fragments of peptidoglycan from *Mycobacterium semeqmatis* or *Escherichia coli* and found that a monomeric fragment N-acetylglucosaminyl (β1, 4) N - acetylmuramyl - L - alanyl - D - isoglutaminyl - meso - diaminopimelyl - D - alanine can replace whole killed mycobacteria or undegraded peptidoglycan Furthermore the terminal N-acetylglucosaminyl and D-alanyl units can be removed without loss of adjuvanticity A synthetic compound, N-acetylmuramyl-L-alanyl-D-isoglutamine turns out to be at least as active as the monomeric unit but a synthetic disaccharide-L-alanyl derivative is inactive So are the hexosamine free di- to tetra peptides Lederer concludes that the glycopeptide linkage and D-isoglutaminyl unit are indispensable for an adjuvant effect

Nauciel and Fleck (*C r Acad Sci Paris*, **276D**, 3499–3500, 1973, *Nature*, **250**, 517–518, 1974, *Eur J Immun*, **4**, 352–356, 1974) disagree on the first point but support the importance of D-isoglutamine They find that the peptide subunit alone possesses the same adjuvant activity as intact peptidoglycan at least in delayed-type hypersensitivity

reactions to azobenzenearsonate-N-acetyl-L-tyrosine
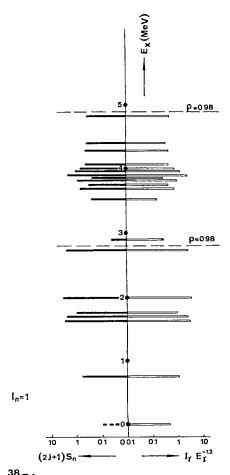
In a search for other points in the immune response at which peptidoglycan might function, the inhibitory effect of this substance on macrophage migration (Heymer *et al*, *J Immun*, **116**, 1743–1754, 1973) is relevant This effect seems to be distinct from migration inhibition of macrophages mediated immunologically by factors secreted by activated lymphocytes A direct action of peptidoglycan on the macrophage surface is possible but little is known as yet about the structural features responsible for this interesting effect

Finally, stimulation of graft rejection —for example of tumour cells—by *Mycobacterium tuberculosis* BCG has been studied by Zbar *et al*, (*J natn Cancer Inst*, **48**, 831–835, 1972), using isolated mycobacterial cell walls The effect is complex and, at least in the systems in which virally induced tumours were studied, stimulation of host interferon production by the mycobacterial adjuvant is possible But this presumably is not involved in host rejection of chemically induced tumours Could a lectin-like activity of antibodies directed against the polysaccharide part of peptidoglycan be involved in host reactions against tumour cells? Several lectins that agglutinate tumour cells (for example, wheat germ agglutinin Allen and Neuberger, *Biochem J*, **135**, 307–314, 1973) interact with an N-acetylchitobiose unit that is found commonly in mammalian glycoproteins, and also bind bacterial peptidoglycan containing an analogous repeating disaccharide unit In some interesting experiments Shier (*Proc natn Acad Sci USA*, **68**, 2078–2082, 1971) prepared antisera against a synthetic antigen containing N-acetylchitobiose hapten groups His finding that these antibodies show some antitumour effects therefore raises the interesting question whether antibodies of similar specificity raised in response to peptidoglycan mimic this reactivity

Single-particle states of nuclei

from P E Hodgson

MUCH of our knowledge of atomic nuclei has been obtained by studies of reactions that remove one nucleon from the nucleus or add one nucleon to it Analysis of the cross sections of these interactions gives the quantum numbers and the occupation probabilities of the single-particle states of the simple shell model This is a very basic feature of nuclei, and it is related to many other nuclear properties such

^{38}Cl

Fig. 1 Comparison between the stripping strengths $(2J+1)S_n$ for the reaction ^{37}Cl(d,p)^{38}Cl to many states of ^{38}Cl (solid lines) and the reduced transition probabilities after thermal neutron capture ^{37}Cl(n,γ)^{38}Cl (open lines) Only the transitions with orbital angular momentum transfer $L_N=1$ are included The (n,γ) partial radiation widths were normalised so that their largest value equals the corresponding $(J+1)S_n$ values The correlation coefficients are given for levels up to 2 8 MeV and for all levels

as their charge and matter distributions, electromagnetic transition rates and quadrupole moments

The theories used to analyse the experimental data, in particular the distorted wave theory, involve certain approximations and are subject to some assumptions that together limit the accuracy of the final results The formalism contains some purely mathematical approximations, and physically there are for example uncertainties connected with the possible contribution of compound nucleus and two-step reaction processes Some improvement in the accuracy of these analyses has recently come from the use of the j-dependent sum rules (*Nature*, **249**, 695, 1974) More recently Clement in a contribution to a conference on nuclear structure and spectroscopy held last year in Amsterdam has drawn attention to the importance of comparisons between the (d,p) stripping and the neutron capture data

The (d,p) deuteron stripping reaction and the (n,γ) neutron capture reaction both deposit a neutron in one of the unoccupied states of the final nucleus, and so both should give the same result for the structure of a particular nucleus The important quantities are the spectroscopic factors that measure the single-particle strengths of the states in the final nucleus and the corresponding transition probabilities for neutron capture Comparison of the numbers obtained for the same nucleus by the (d,p) and (n,γ) reactions is thus a good test of the reliability of both analyses

Such a comparison was made for ^{38}Cl by Spits and Akkermans (*Nuclear Physics,* **A215**, 260, 1973) They measured the cross section for the ^{37}Cl(n,γ)^{38}Cl neutron capture reaction to many states of ^{38}Cl and compared the transition probabilities they obtained from their data with the spectroscopic factors found from the corresponding ^{37}Cl(d,p) ^{38}Cl deuteron stripping reaction by Engelbertink and Olness (*Phys Rev*, **C5**, 431, 1972) The results are compared in Fig 1 and show a remarkably high degree of correlation between the two sets of numbers Indeed if the data are analysed statistically the correlation coefficient between the two sets is as high as 98%

Similarly high correlations have now been obtained for a whole series of nuclei, a few cases of low correlation being readily explicable as due to the presence of nearby resonances The result for ^{38}Cl is thus not an isolated example, but is quite typical

These astonishingly high correlations between spectroscopic factors obtained by quite different reactions validate at a stroke essentially all the significant approximations and assumptions in the theories of deuteron stripping and neutron capture (Kopecky, Spits, and Lane, *Phys Lett*, **49B**, 323, 1974) The reaction mechanisms are so different that it is inconceivable that any perturbing factor in the analysis should have the same effect in the two reactions The excitation energies of the compound nuclei in the two cases differ widely, so the compound nucleus cross sections must be different and since they do not affect the results they must both be small Similarly for the two-step contributions (*Nature*, **247**, 179, 1974) which must be quite different in the two types of reaction All perturbing factors, if important, could only reduce the correlation coefficients and since they are so high the analysis provides strong evidence that both sets of analysis are rather more accurate than has been generally believed This very satisfactory result should be a welcome stimulus to further analyses of single particle states in nuclei

New method of determining nuclear quadrupole moment

from P E Hodgson

THE recent theoretical work of Clement (*Nuclear Phys*, **A213**, 492, 1973) on the nuclear shell model has made it possible to express the nuclear quadrupole moment in terms of single particle quadrupole integrals and the spectroscopic factors extracted from single proton transfer reactions In some cases all the required data are available and the resulting values of the nuclear quadrupole moment are in excellent accord with those determined by atomic methods

In a previous note (*Nature*, **249**, 695, 1974) the work of Clement and Perez on the j-dependent sum rules was described This shows how the spectroscopic factors for stripping and pickup reactions on the same nucleus can be related to each other, thus providing multiple checks of the values obtained for the spectroscopic factors and confirming the validity of the distorted wave theory used to obtain them If all the necessary data are available, the spectroscopic factors can be obtained with an accuracy of better than 10%

The single-particle quadrupole integrals are simply overlap integrals of two single-particle wavefunctions with the quadrupole operator These may be evaluated very easily using harmonic oscillator wavefunctions, but the results are rather inaccurate, especially for valence nucleons Much better results can be obtained with single-particle wavefunctions evaluated as the eigenfunctions of a Saxon–Woods potential with parameters adjusted to give the correct binding energies, these can now be obtained easily with an electronic computer

Thus for nuclei for which a complete set of data are available it is possible to use the formalism of Clement to calculate the quadrupole moment

Clement and Perez (*J Phys*, **A7**, 193, 1974) have now done this for ^{37}Cl, and find $Q = -0\ 057 \pm 0\ 005$ barn which compares very well with the atomic value $Q = -0\ 062$ barn, showing the reliability of the method As more spectroscopic factors become available this should become a powerful method of determining nuclear quadrupole moments, which will be especially useful in cases where the atomic value is not easily obtained

Diode molecules

from our Moleculai Physics Correspondent

NOT many years ago a distinguished theoretical chemist deliberately left the field, explaining that an important factor in his decision to work elsewhere was the strong possibility, as he conceived it, that no further, totally new and astonishing phenomenon might ever again be discovered at the purely molecular level Premature as this may have been at the time, and counter-indicated as it may seem by subsequent events—for example in the area of laser photochemistry—it remains broadly true that, in spite of remarkable advances in instrumentation and the mastering of known effects in ingenious ways, the incidence of altogether new ones in the molecular world could be said to have lagged behind the steady stream of named, prizewinning, conference-engendering effects in other subjects Having said which, one may as reasonably remark that refuge from pessimism can be sought in the ill-defined yet compelling suspicion that what does remain to emerge from the fastnesses of undiscovered molecular behaviour may prove to be very spectacular indeed

A sound conjecture—because in a way a truism—is that radically new effects are likely to involve some form of electromagnetic, probably time-dependent phenomenon Stimulated emission fitted this description, the widely publicised speculations about molecular superconductivity are a long shot in the same direction where the merest vestige of an effect would be altogether sensational

Closer to Earth, yet of undoubted promise, is the recent speculation by Arieh Aviram and Mark Ratner (*Chem Phys Lett*, **29**, 277, 1974) that it should be possible to design molecular rectifiers by careful patching together of suitable donor and acceptor subsystems The key to this idea is that, by bridging an acceptor unit, perhaps a quinone-like ring system, by a σ-bonded hydrocarbon bridge to an electrophobic unit, perhaps containing methoxy-groups, the necessary current–voltage asymmetry could be achieved Actually more exotic candidates for donor and acceptor are available and might be tailor-made for the task, with the passive bridge playing an essential part both in providing rigidity and keeping donor and acceptor at arm's length —just out of range of direct level-interactions, though not of tunnelling transfer

No doubt these bare ideas will need considerable testing and elaboration before it can be claimed that rectifier action is a practical possibility But, showing admirable willingness to back their level diagrams with some real mathematics, Aviram and Ratner go on to present a careful perturbational calculation of the current–voltage characteristics of a plausible rectifier structure (actually a simple hemi-quinone) This is semi-quantitative quantum mechanics at its best, using relatively crude approximations to warrant an idea and point to further refinements When the necessary matrix-elements are computed, with reasonable assumptions of ionisation energy and electron affinity, the I–V characteristic is indeed of the form predicted, with an appreciable asymmetric barrier to conduction

Needless to emphasise, far too much is left to good fortune for the results to approach a theoretical 'proof' of viable rectifier action Direct electrode-electrode tunnelling could swamp the whole effect, the complex problems of metal–organic junctions and crystal field effects have yet to be built in— each might either enhance or kill off the desired effect Yet the work described has a convincing air of the *premier pas qui coûte* and we may well hear a lot more of the molecular rectifier, perhaps the absolute ultimate in electronic miniaturisation

Volcanoes and ice ages

by John Gribbin

ACCORDING to Kennett and Thunell (*Science*, **187**, 497, 1975) "volcanic ash in deep-sea sections indicates very high rates of explosive volcanism during the last two million years" This apparent global increase in Quaternary explosive volcanism flies in the face of some cherished geophysical beliefs—but it offers a new insight into the widespread and frequent glacial activity that has occurred over the same period

Simple tectonic theory would suggest that volcanic activity is related to local events—collisions between blocks of crust which lead to mountain building But it now seems from study of 320 deep-sea sections drilled around the world during the Deep Sea Drilling Project that there has been a much higher rate of explosive volcanism from both island arc and 'hot spot' volcanoes during the past 2 million years than the 'normal' level of the past 20 million years And, as Kennett and Thunell point out, "increased Quaternary volcanism coincides approximately with that episode of the Cenozoic marked by major and rapidly fluctuating climatic change"

Just how these two effects may be related remains to be unravelled, but several interesting possibilities are raised First, it is well established that under appropriate conditions the spread of volcanic dust in the atmosphere caused by a marked increase in volcanic activity could trigger the onset of an ice age The dust simply acts as a veil blocking some of the Sun's heat, and if the resulting cooling lasts for long enough for ice cover to become established it may take more than the removal of the dust veil to melt the ice, because the ice's high albedo will continue to ensure that little solar heat is absorbed

But studies of shorter stretches of the geological record than the 20 million year sample of the DSDP show that although widespread volcanic activity often precedes the spread of the glaciers it is possible either for glaciation to occur without any increase in volcanic activity, or for there to be an increase in volcanism without immediate climatic repercussions This encourages speculation that there are one or more additional mechanisms of key importance in triggering ice ages The Milankovitch model, in which phases of cooling are produced by changes in the orientation of the Earth and by 'stretch' of its orbit around the Sun is perhaps the most attractive of these mechanisms, especially in the form in which Kukla has recently presented it (*Nature*, **253**, 600, 1975) To my mind, the most plausible overall model of ice ages is that when the northern hemisphere is at its coolest phase of the astronomical Milankovitch cycle, conditions will be appropriate for ice cover to develop dramatically if some further effect—such as volcanic activity—provides the final trigger

But the model can also be turned on its head The repeated loading and unloading of the Earth's crust caused by the advance and retreat of the glaciers might, in fact, trigger increased volcanic activity And, if each of these processes occurs there could be a snow-ball effect by which a small climatic fluctuation becomes a full-scale ice age

Whatever the truth of the matter, these new discoveries emphasise the impossibility of trying to deal with problems in the earth sciences within rigid compartments The development of plate tectonic theory has already contributed greatly to the study of past climates by explaining how the present state of the globe, with a nearly land-locked North Polar Ocean, differs from the situation in earlier times when the continents were distributed differently and ice ages were extremely uncommon (see page 14 of this issue of *Nature*) And at a more immediately important level, it seems that a better understanding of volcanism, as well as a better understanding of ocean currents and other aspects of earth science, will be an essential prerequisite to a really satisfactory theory of climatic change

articles

Tilt and strain monitoring of the 1974 eruption of Mount Etna

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Using advanced modern equipment capable of continuous monitoring it has been possible to measure the degree of tilt and strain undergone by Mount Etna during a recent phase of volcanic activity The results are open to interesting interpretation, and the model suggested here, though hypothetical, is quite plausible geologically

THE measurement of tilt and of distance have proved valuable techniques in the surveillance of volcanic activity[1] The interpretation of the observed deformation associated with a small eruption in January–March, 1974, gives some insight into the eruptive mechanisms operative on Etna, and also provides some indication of the potential of geodetic techniques for the prediction of activity on that mountain

The tilt transducer used in this study was an Autonectics biaxial borehole tiltmeter consisting essentially of a circular spirit-level mounted at the bottom of an aluminium alloy tube (Fig 1) Tilt changes were monitored using a pair of a c resistance bridges which gave an output proportional to the position of the bubble in two orthogonal axes These voltages were recorded on a two channel Rustrak chart-recorder with bias-steppers which gave an effective increased chart width[2] The system was powered by alkaline-manganese cells, and to conserve power the tiltmeter was run for three 1-min intervals in every hour Sampling intervals were timed by an Accutron clock and the time scale of the record was derived by dividing the length of the record by the total number of days elapsed

The tiltmeter was installed in a borehole 1 5 m deep in ash (Fig 2), 3 km south of the Central Crater The instrument was levelled using an oscilloscope with its horizontal and vertical inputs connected to the tiltmeter outputs, the top of the tiltmeter tube was then moved manually to centralise the spot on the screen[3]

On January 30, 1974 a small flank eruption began on the western side of Etna[4], and during two eruptive periods (January 30–February 16 and March 11–29) produced two small pyroclastic cones and approximately 6×10^6 m^3 of lava (Fig 2) The correlation between the tilt record and this eruptive cycle is clear, detailed and apparently free of spurious effects The following volcanological interpretation is based on the assumption that a positive change in the radial component indicates inflation of the summit region The records of both the radial and tangential components of tilt during the 10 months of monitoring show very similar patterns of change (Fig 3)

The tilt record of the period before January 1974 is very quiet, but about three weeks before the eruption (the two phases of which are represented by 1-2 and 3-4 in Fig 3), the summit inflated and a small but regular fluctuation of tilt began Two weeks before the eruption started the radial component recorded a series of very sharp though small deflations (Fig 3ci) which gave way to a series of large amplitude fluctuations (Fig 3cii) up to and immediately following the start of eruptive activity The onset of the initial deflations corresponds closely with the start of the seismic activity that was felt at the volcano, and is interpreted as an expression of the initiation of fracture propagation from the magma reservoir at the summit to the eruption site on the western slopes The large amplitude tilt fluctuations (Fig 3cii) are cycles of rapid inflation followed by deflation of the summit and could represent the pulses of magma moving into the flanks of the volcano Careful examination of the record shows that in most cases deflation was more rapid than the inflation, particularly for the tangential component It may be possible

Fig 1 Diagrammatic cross section of the tiltmeter installation

Fig 2 Sketch map showing the location of the tiltmeter with respect to the eruption site and the Central Crater
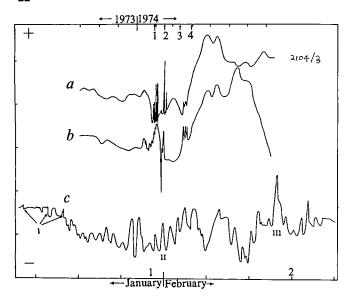
Fig 3 Tiltmeter record for the period September 28, 1973–August 8, 1974 *a, b*, Smoothed traces of the record (sampled at weekly intervals), for the whole period, indicating the tilt variations in directions radial (*a*) and tangential (*b*) to the Central Crater at the summit of Etna *c*, The non-smoothed trace of the radial component during and just before the first phase of the eruption The scale of the abscissa for the two curves *a* and *b* is at monthly intervals, for *c* at 5-d intervals The interval of the ordinate is 50 m rad (equivalent to a change in elevation of 5 cm km^{-1}), and the positive sense of tilt change shown is summit up (*a*), and ENE up (*b*)

to correlate these magma pulses with specific events in the vent activity The particularly large tilt changes just before the end of the first phase of activity (Fig 3*ciii*) coincided with a brief rejuvenation of effusive activity which produced three new lava flows[4]

A period of calm characterised the tilt record for the interval between the two eruptive episodes during which the summit remained deflated The degree of tilt that marked the start of the second phase was relatively minor compared with that of the first phase and is interpreted as indicating that the magma moved from the summit along the same conduit as that formed during the previous activity The marked decline in vent activity and the rapid, smooth summit inflation on March 23 presaged the closing of the conduit and the end of the eruption

The summit inflated throughout April and early May and then remained stable to the end of the record The gradual tilt change in the tangential component during July and August 1974 cannot be interpreted as yet The sense of this change—west(eruption) side down when the summit rose and vice versa—is unexpected, but could be because deflation of the western slopes complemented the inflating summit[5]

The most striking feature of the record is the consistent frequency of the tilt fluctuations—one fluctuation a day—during and just before the eruption, a feature that is not evident during the non-eruptive periods It suggests some non-volcanic causal mechanism such as, possibly, earth tides

Since October 1971, precise electromagnetic measurements of distance have been made periodically on a network of 19 permanent benchmarks arranged in 2 concentric rings around the summit of the volcano (Fig 4) Early surveys were made with the National Physical Laboratory Mekometer III prototype[6] but since 1973 a Hewlett-Packard 3800B has been used

Up until June 1973 the measurements showed changes of up to 310 mm in the lengths of the lines on the summit network They are interpreted as showing that the summit region was inflating elastically[7], although the Mogi model[8]—used to explain the deformation of Kilauea[9]—does not agree with the observations The model which best describes the observations is that in which a vertical cylinder of infinite length but finite diameter expands in an elastic half-medium[7,10] The displacements of all of the points in the half-medium are inversely

proportional to the square of their distance from the cylinder axis and are directed radially outwards from it The length changes observed up until June 1973 gave excellent best-fit agreement with the changes which could be expected were such a cylinder 200 m in diameter located beneath the North-east Crater (Fig 4) Exceptions to this model occur on the lines involving stations 9, C1 and 13 (Fig 4) which are situated on very recent flows and seem to have moved downslope after the apparent solidification of the lava

The changes observed on the summit network between June 1973 and August 1974 (Fig 4) do not fit such a simple model as the earlier changes

Theoretical models

The model which best fits these results involves two cylinders one expanding and situated as before beneath the North-east Crater, and the other—of the same diameter—contracting and centred on the Chasm (the figures given by the model are shown in brackets in Fig 4) The contracting cylinder would not affect the lines on the eastern side of the NE–SW tranding fissure, the most important tectonic feature of the summit area[11] Anomalous movements again affect stations 9, C1 and 13, and the probable displacement vectors are shown in Fig 4 Similarly, station 12 seems to have moved anomalously, though this is not understood

The complexity of this model cannot be denied, but the volcanological interpretation of the cylinders is simple
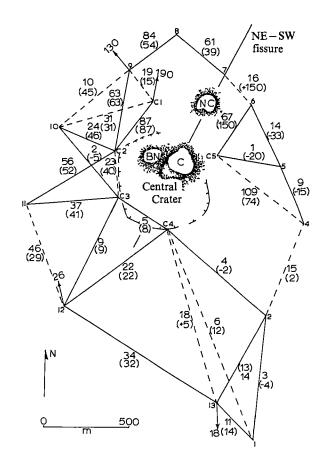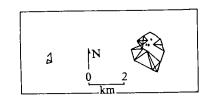
Fig 4 The summit region of Mount Etna C, The Chasm, BN, the Bocca Nuova—both of which are surface expressions of the Central Crater, NC, the North-east Crater Geodetic benchmarks shown by small numbers Solid lines, distances that increased, broken lines, those that decreased, unbracketed numbers, the observed changes of distances (mm) from June 1973 to August 1974 Bracketed numbers, changes (mm) expected from the model described in the text Displacement vectors for the four anomalous stations are also shown
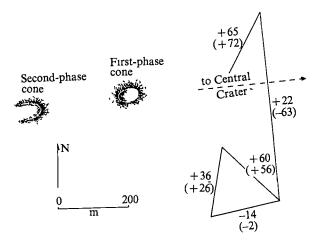
Fig. 5 The site of the January–March, 1974 eruption on the western flank of Etna, showing the first phase (January–February) cone, the second phase (March) cone, and the geodetic benchmarks Unbracketed numbers, distance changes (mm) observed between February and August 1974, bracketed numbers, changes of length (mm) expected from the model described in the text Inset, geography of the two networks

The continuous effusive activity which characterised the North-east Crater for many years stopped abruptly during the 1971 eruption[11] It thus seems that before 1971 the North-east Crater had acted as a safety valve to the continuous movement of magma from below but that since then pressure has built up beneath a blockage in the conduit

In contrast, the contracting cylinder part of the model indicates the reverse process, the decrease of pressure on the western side of the Central Crater, caused by the removal of magma from the conduit beneath the Chasm

During the February phase of the 1974 eruption precise distance measurements were made on a small network of benchmarks in the immediate vicinity of the eruption site, they were repeated in August 1974 The changes between the measurements are shown in Fig 5 as unbracketed numbers A contracting cylinder model like that applied to the Chasm,

but of 50m diameter, situated beneath the first-phase cone agrees quite well with the observed changes This can be improved, however, by including the influence of a horizontal cylinder which joins the first-phase cone to the Central Crater and which contracts by one-fifth the amount of the vertical one The observed and expected changes of line 1–3 disagree, however (Fig 5)

How the models fit

The vertical cylinder could represent the first phase vent, and the horizontal cylinder the feeder conduit from the Central Crater to the eruption The contraction of the cylinders indicates the decrease of magmatic pressure which accompanied the cessation of effusive activity The anomalous change in length of line 1–3 indicates that the feeder conduit (which passes directly beneath the line) widened inelastically during the second phase of the eruption

The deflation of the western side of the summit region and of the horizontal cylinder used to model the deformation to the east of the eruption site suggests strongly that the eruption was fed by a conduit (dyke?) from the Central Crater This view is endorsed by the evidence of a narrow line of micro-earthquakes along the proposed path of the conduit during the activity (G Luongo, personal communication) and by the sympathetic behaviour of the Bocca Nuova[12] which suggests that the conduit originated there

The 1974 eruption seems to have relieved the stress on the western side of the Central Crater but not that to the north around the North-east Crater, the continued inflation in that area must make it a likely place for renewed activity In fact, since the last measurements were made quiet effusive activity has broken out at the foot of the North-east Crater (October 1974)

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Magnetic field in the intergalactic region

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An analysis of the redshift dependence of the rotation measures (RM) and of the intrinsic position angles of the polarised radiation of radio galaxies and QSOs is made The results place an upper limit of 10 rad m^{-2} to the observed RM, coming from the contribution of the magnetic field in the intergalactic region

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FARADAY rotation in a plasma produces a rotation of the plane of linear polarisation of the radio emission, the rotation increasing proportionately to the square of the wavelength The proportionality factor is called the rotation measure (RM), and depends on the product of electron density, magnetic field, and path length inside the plasma The plasma in the radio source gives rise to the source RM Any intergalactic plasma will also contribute, the extragalactic RM is defined here as the observed RM minus the contribution from our

Fig 1 The redshift dependence of $F(q_0,z)$ for various values of the cosmological deceleration parameter q_0 Numbers on curves indicate values of q_0

Galaxy It follows that, setting z_s as the source redshift,

$$\text{RM(observed)} = \text{RM(our Galaxy)} + \text{RM(extragalactic)} \quad (1)$$

$$\text{RM(extragalactic)} = \text{RM(intergalactic)} + \frac{\text{RM(source)}}{(1+z_s)^2} \quad (2)$$

Values of RM(observed) and RM(extragalactic) will be reported elsewhere by myself and P P Kronberg There are two possible ways in which a uniform component of an assumed intergalactic magnetic field can make a contribution to the extragalactic RM of a distant radio source

If the smooth intergalactic density of thermal electrons throughout the observable Universe is sufficiently large, then each volume element along the line of sight to the radio source will contribute to the extragalactic RM, weakened by the factor $(1+z)^2$, where z is the redshift of the volume element So if the lines of sight to two QSOs at different redshifts are very close to each other, they will have a different amount of their extragalactic RM coming from the intergalactic magnetic field

If the intergalactic electron density close to a radio source is much larger than the smooth component permeating the observable universe, then the intergalactic magnetic field close to each radio source will contribute a part to RM, but weakened by the factor $(1+z_s)^2$, where z_s is the radio source redshift

A significant contribution from a random intergalactic magnetic field would contradict the evidence of the least depolarisation occurring with the largest linear and angular sizes of radio sources, as shown by Strom[1,2]

The uniform model

Such a contribution to the extragalactic RM coming from any homogeneous component of an intergalactic magnetic field in a uniform model of the universe is given by

$$\text{RM (intergalactic)} = \int_0^{R_\text{source}} A\cos\theta n_e(R)B(R)(1+z)^{-2}\mathrm{d}R \quad (3)$$

where $A = 8 \ 1 \times 10^5$ rad m^{-2} cm^3 gauss^{-1} pc^{-1}, n_e = electron density (cm^{-3}), B = magnetic field (gauss), θ = angle between direction of B and line of sight, z = redshift of volume element at R, and $\mathrm{d}R$ = element of path length for the radiation coming from the source

From the field equations[3] for zero-pressure models and

$$\Lambda{=}0, \quad \mathrm{d}R = -c\mathrm{d}t = cH_0^{-1} \ \mathrm{d}z(1+z)^{-2}(1+2q_0z)^{-\frac{1}{2}} \quad (4)$$

where c = speed of light, H_0 = Hubble constant, t = cosmic time, and $q_0 =$ deceleration parameter of the Universe

In a homogeneous universe, it is plausible to use the assumptions that

mean matter density $\rho \sim (1+z)^3$
mean electron density $n_e \sim \rho \sim (1+z)^3$
magnetic field $B \sim \rho^{2/3} \sim (1+z)^2$ "frozen-in"

Integrating equation (3)

$$\text{RM (intergalactic)} = K\cos\theta F(q_0, z_s) \quad (5)$$

where $\quad K = An_{e_0}B_0(c/H_0)$

and

$$F(q_0z) = [(1+2q_0z)^{3/2} + (6q_0-3)(1+2q_0z)^{1/2} + (2-6q_0)]/6q_0^2$$

and the subscript zero refers to the present epoch An estimate of the numerical value of the quantity K is about 5 rad m^{-2} (assuming $n_{e_0} = 10^{-6}$ cm^{-3}, $B_0 = 10^{-9}$ gauss, $H_0 = 50$ km s^{-1} Mpc^{-1}) The function $F(q_0,z)$ is plotted in Fig 1 for $0 \leqslant z \leqslant 4$, and for q_0 values of 0, 1/2, 1, 2, 3 and 10 $F(q_0,z)$ varies between 0 and 12 for radio sources in this range (A mean density of the Universe of 3×10^{-30} g cm^{-3} corresponds to $n_e \sim 2 \times 10^{-6}$ cm^{-3}, assuming 100% ionisation and 100% hydrogen)

I have used a numerical technique to fit the equation
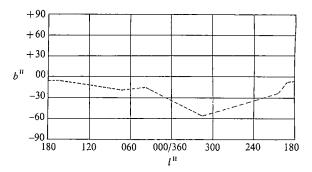
$$\text{RM (extragalactic)} = KK\,F(q_0,z) + A(3) \pm A(4)/(1+z)^2 \quad (6a)$$

to the data where $KK = K\cos\theta = A(1)$ (rad m^{-2}) and $q_0 = A(2)$ $A(3)$ is a constant residual galactic contribution (rad m^{-2}) and $A(4)$ is the average positive source RM (rad m^{-2}) Because of the smoother magnetic field below the galactic plane[4] I used as input data the extragalactic RMs of 44 QSOs and galaxies with known redshift in approximately half of the South Galactic Hemisphere, as will be discussed elsewhere (J P V and P P Kronberg, unpublished)

This chosen region (Fig 2) lies between the dashed line and the South Galactic Pole It was arrived at by fitting a slab model to represent the galactic magnetic field contribution to the observed RM of discrete radio sources In so doing, the RM with large absolute values (>150 rad m^{-2}) were removed, because of their effect on the least squares fitting technique used We also selected sources with a redshift greater than 0 054 to eliminate unidentified galactic sources as well as to take advantage of the Doppler effect on source RM, enabling the galactic (local) contribution to RM to become more significant The best fitted slab is not necessarily parallel to the galactic plane, and sources not seen through the slab were not used to determine the slab parameters The boundary defining the input sources actually used in the best fitted slab corresponds to the dashed line in Fig 2, enclosing the South Galactic Pole

For all galaxies and known QSOs below the dashed line with observed RM, the galactic contribution to RM was computed and removed First, assuming that cos θ = constant for the redshifted sources below the dashed line, it was found that q_0 varied significantly when using different selection criteria on the input data

Fig 2 Projection of the sky in galactic coordinates, in which the region of the South Galactic Hemisphere studied in the two models discussed in the text is shown below the dashed line
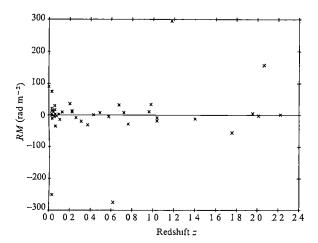
Fig 3 Plot of the extragalactic rotation measures as a function of the redshift *z* for the 44 QSOs and galaxies studied in the two models

So the approximation $\cos\theta$ = constant was rejected and θ computed for each radio source (assuming a given direction in the sky for the intergalactic magnetic field—see below) with as input data the extragalactic RM divided by $\cos\theta$ The fitting function used was

$$RM \text{ (extragalactic) } /\cos\theta = KF(q_0,z) \qquad (6b)$$

where K and q_0 are to be fitted to the input data

Many trials were made, using three different directions for the assumed intergalactic magnetic field Only a direction towards the spiral arm magnetic field ($l^{II} = 90°$) gave results that were not changed significantly when different selection criteria for the input data were used This can perhaps be explained by a small residual contribution (or overcorrection) from the magnetic field in the galactic spiral arm

As Fig 3 shows, the sample contains about an equal number of sources with positive and negative values of extragalactic RM If there is a sufficiently large contribution from the intergalactic region, then the quantity on the left hand side of equation (6b) should be of the same sign for all the radio sources (except for strong source RM) A plot of this quantity as a function of redshift also shows, however, about an equal number of sources with positive and negative values

So an increase of extragalactic RM with increasing redshift seems ruled out within the errors permitted by the data A useful upper limit to the extragalactic RM from the uniform model would be about 10 rad m^{-2} (Fig 3) This value can be halved if a small residual galactic contribution is accepted ($A(3)$ in equation (6a)) A plausible alternative to the uniform model would be to say that any real contribution from an intergalactic magnetic field would originate near the radio source, instead of in a region far away from a radio source where the mean matter density and the ionisation rate are both likely to be smaller This is the clumpy model

The existence of intergalactic material in clusters of galaxies has often been proposed De Vaucouleurs *et al*[5] presented evidence for intergalactic extinction in the light of normal optical galaxies located close to the plane of the supergalaxy (centred on the Virgo cluster of galaxies) Miley *et al*[6] proposed that the "head-tail" radio galaxies represent the radio trails of the trajectories of active radio galaxies through a dense intergalactic medium within clusters of galaxies In trying to evaluate the contribution to the source rotation measure coming from an ionised intergalactic plasma, a major problem arises because the geometrical distribution of this plasma and the actual position of the radio source in the cluster are not known *a priori*

To facilitate the computations I shall make several assumptions First, the existence of a uniform component of an

intergalactic field Second, that the ionised intergalactic plasma, within several megaparsecs of a radio source, is denser than the possibly smooth component permeating clusters of galaxies or even the Universe Third, a geometrical distribution originating in the following form

Let a radio source at redshift z_s eject n_e electrons cm^{-3} up to a source distance $\Delta R/2$ perpendicular to the intergalactic magnetic field B (no limit in the direction parallel to B) A subsequent diffusion along the lines of B may occur to equalise n_e in this cylindrical model with the radio source on the axis

Provided that the products of n_e,B, and ΔR are within an order of magnitude for most of the radio sources studied, and that the overall angular area in the sky is sufficiently small, the slab model (see above) can test for the existence of the ordered component of an intergalactic magnetic field The input data consist of the extragalactic RM transferred into the source frames by

$$RM \text{(extragalactic)} = \frac{RM\text{(source)} + RM\text{(intergalactic)}}{(1+z_s)^2} \qquad (6c)$$

Table 1 Rotation measures in the source frames, after removal of the contribution from our Galaxy, for use in the clumpy model

Name	RA, dec	RM (rad m^{-2})
3C2	0003−00	−37
3C9	0017+15	−24
3C15	0034−01	−4
3C17	0035−02	+13
PKS	0038−02	+1394
PKS	0043−42	−6
PKS	0045−25	+91
3C29	0055−01	+10
PKS	0056−00	+22
3C33	0106+13	+2
3C37	0115+02	+88
PKS	0119−04	+38
PKS	0131−367	−1
3C47	0133+20	+1
3C48	0134+32	−60
PKS	0155−10	−719
PKS	0226−038	+1472
PKS	0237−23	+12
3C76 1	0300+16	−12
3C78	0305+03	+14
3C79	0307+16	−14
3C88	0325+02	+22
3C94	0350−07	+40
3C98	0356+10	+79
PKS	0403−13	−14
3C109	0410+11	−35
3C135	0511+00	+11
3C138	0518+16	−88
PKS	0521−36	−40
3C403	1949+02	+19
PKS	2058−28	+17
PKS	2115−30	+132
3C433	2121+24	−18
PKS	2135−14	+51
PKS	2209+08	+16
3C442	2212+13	−1
3C445	2221−02	+33
3C446	2223−05	−71
PKS	2230+11	−78
PKS	2247+11	+4
3C454	2249+13	−427
3C459	2313+03	+20
3C465	2335+26	−267
PKS	2356−61	+3

The same radio sources used in the uniform model were used for analysis of the clumpy model, RM(source)+RM(intergalactic) are listed in Table 1

The best results occur when radio sources with known redshifts are separated according to redshift QSOs at $z > 0.5$ and with RM(source) less than 150 rad m^{-2} yield a direction for a possible magnetic field that is almost parallel (within the errors) to the spiral arm magnetic field in the Southern Galactic Hemisphere It is possible that not all the galactic contribution had been removed from the observed RM because of the idealised

model of a slab, this effect showing up at large z because of the Doppler shift affecting RM(source) Thus, for QSOs at intermediate and large z, the residual galactic contribution is as big or bigger than the extragalactic RM

For radio sources at redshifts closer than 0 5 and with RM (source) less than 150 rad m^{-2}, QSOs and galaxies taken together or separately yield the best results for a magnetic field direction $l^{II} = 200°$, $b^{II} = 0°$, with an error of 40° in both coordinates The parameter K is about -7 rad m^{-2} If we assume an intergalactic electron density n_e close to a radio source in the range 10^{-4} to 10^{-5} cm^{-3}, and a value for ΔR in the range 10^6 to 10^8 pcs, we find that this value for K corresponds to a field strength in the range 10^{-6} to 10^{-9} gauss This is to be compared with the upper limit of about 10^{-7} gauss set by Zel'dovich[7] on the basis of an isotropic cosmological model concerning nuclear reactions But in view of the strong assumptions used in the clumpy model, one cannot state that this last result constitutes a proof for the existence of an intergalactic magnetic field

The intrinsic position angle of linear radiation is determined by the orientation of the magnetic field inside a radio source It is altered neither by the intervening medium between the radio source and the Earth, nor by the Doppler effect Some theoretical models of radio galaxies and QSOs (see ref 8) presuppose the existence of an intergalactic magnetic field in the region of space where a radio galaxy or a QSO occurs These models have the rotational axis of a galaxy orthogonal to the direction of the intergalactic magnetic field Magnetic

"tongues" erupt along the rotational axis, and radio emission commences with the ejection of relativistic electrons into the tongues, through the synchroton process, it is polarised with the electric vector parallel to the direction of the intergalactic magnetic field (for young radio sources) According to Piddington's model[8] neighbouring sources may therefore have similar E–vector orientations

I have plotted the 114 redshifted QSOs and galaxies with known RM in the Southern Galactic Hemisphere[9] on Mercator maps, each radio source in its proper redshift range The redshift ranges were $0 < z < 0 1$, $0 1 < z < 0 2$, $0 2 < z < 0 3$, $0 3 < z < 0 5$, $0 5 < z < 0 7$, $0 7 < z < 1 0$, $1 0 < z < 1 5$, $1 5 < z < 2 3$ The median number of sources on these plots was nine A qualitative study of the intrinsic position angles shown on these plots in various redshift ranges failed to indicate any parallelism, even for neighbouring sources in the sky

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A gene cluster in *Aspergillus nidulans* with an internally located *cis*-acting regulatory region

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Work reported here on the fungus Aspergillus nidulans *has provided the first definitive demonstration of operon-type organisation in an eukaryote genome It has been shown that the* prnA *and* prnB *genes concerned with proline metabolism form a gene cluster with the regulatory region lying between the two putative structural genes* prnA *and* prnB *Regulatory mutations* (prn^d) *probably leading to relief of carbon catabolite repression, map in between* prnA *and* prnB *and are* cis-*dominant with respect to both The properties of these regulatory mutations and other findings suggest that carbon catabolite repression may be mediated by a negative control system in* A nidulans *This gene cluster is particularly interesting in view of its divergent orientation (with the regulatory region located in the centre of the operon) and for the fact that unlike the divergent operons known in prokaryotes, the divergent orientation is related to the way in which this particular operon may be regulated*

IN the ascomycete fungi, one of the most genetically well characterised groups of eukaryotes, clustering of functionally related genes is relatively rare It is generally confined to

instances in which the clustered genes code for enzymes which form aggregates (refs 1 and 2 and D J Cove, H N Arst, and C Scazzocchio, in preparation) None of the few clusters which have been reported has been demonstrated to contain an internal control region such as that described[3,4] in the *arg*ECBH cluster of *Escherichia coli*, where transcription proceeds divergently[5,6] Indeed, there has been no convincing demonstration of a *cis*-acting control region associated with a fungal gene cluster at all

In *Saccharomyces cerevisiae*, however, there are several reports of *cis*-dominant operator constitutive type mutations adjacent to negatively controlled structural genes[7-10] In *Aspergillus nidulans* (D J Cove *et al*, unpublished) and *Neurospora crassa*[1], the preponderance of positive control systems weighs heavily against the probability of obtaining *cis*-acting regulatory mutations Initiator constitutive mutations should be rare and the more frequent initiator negative mutations will be difficult to distinguish from negative mutations in adjacent structural genes under their control New and powerful selective techniques have resulted, however, from an extensive study[11] of nitrogen metabolite repression (ammonium repression of the syntheses of enzymes involved in nitrogen metabolism) in *Aspergillus nidulans* The product of a gene designated *area* is likely to be a positive control element which allows the synthesis of a large number of enzymes and permeases involved in nitrogen metabolism One class of mutations, designated *area^r*, leads to loss of these enzymes and permeases and a
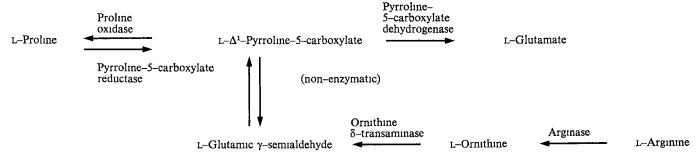
Fig. 1 Pathways of proline, ornithine, and arginine catabolism

consequent inability to utilise a wide variety of nitrogen sources other than ammonium Presumably, *areA*ʳ mutants lack a functional *areA* product Given the pleiotropic nature of the *areA*ʳ phenotype, suppressor mutations affecting the regulation of a single enzyme or a group of enzymes having a common regulatory element can readily be detected For example, mutations which relieve carbon catabolite repression have been obtained because they enable *areA*ʳ strains to utilise those nitrogen sources catabolised by carbon catabolite-repressible enzymes[11,12]

Here we present evidence that the regulation of proline catabolism in *A nidulans* involves three distinct forms of control induction, nitrogen metabolite repression, and carbon catabolite repression

Moreover, a putative structural gene for a proline permease is tightly linked to a putative structural gene for a component common to proline oxidase and Δ^1-pyrroline-5-carboxylate dehydrogenase Between these two genes is a region in which *cis*-acting regulatory mutations affect the expression of both

Control of proline transport and catabolism

The relevant pathways[13-17] of proline and ornithine catabolism are shown in Fig 1 Control characteristics of proline transport,

Table 1 L-Proline transport by wild type and mutant strains

Relevant genotype or calculation	Specific transport activities using mycelia grown in media with							
	1% D-glucose as C				0 3% D-glucose as C			
	595 µM uric acid		10 mM NH₄⁺		595 µM uric acid		10 mM NH₄⁺	
	−	+ 5 mM L-proline	−	+ 5 mM L-proline	−	+ 5 mM L-proline	−	+ 5 mM L-proline
Wild type	0 80	1 63	0 42	1 26	0 95	2 80	0 27	2 40
prnA-1	0 76	1 65	0 50	1 40	—	—	—	—
prnB-6	0 58	0 70	0 12	0 15	0 50	0 66	0 08	0 30
prnA-1 prnB-6	0 60	0 63	0 15	0 17	—	—	—	—
*prn*ᵈ-20	0 40	1 80	0 70	1 90	1 50	3 50	0 30	4 70
*prn*ᵈ-20 *prnB-36*	0 12	0 28	0 14	0 40	0 14	0 30	0 06	0 14
*creA*ᵈ-1	0 80	2 50	0 50	1 86	0 78	2 80	—	—
Corrected wild type	0 22	0 93	0 30	1 11	0 45	2 14	0 19	2 10
Corrected *prn*ᵈ-20	−0 18	1 10	0 58	1 75	1 00	2 84	0 22	4 40
Corrected *creA*ᵈ-1	0 22	1 80	0 38	1 71	0 28	2 14	—	—

Fourteen mutants unable to utilise proline as a nitrogen source were isolated after N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis[26] of a strain of genotype *yA-2 proA-6 puA-2* (yellow conidial colour, proline-requiring, putrescine-requiring), using the putrescine starvation selection technique[27] On the basis of growth responses (Tables 4 and 5) and complementation tests, eight of these were designated *prnA*⁻ and the other six *prnB*⁻ *prnA*⁻ and *prnB*⁻ mutations are recessive and complement with each other in heterokaryons and diploids Most further work has been done with *prnA-1* and *prnB-6* The tight linkage of *prn*ᵈ mutations to *prnA* and *prnB* makes it impracticable to obtain *prn*ᵈ *prnA*⁻ and *prn*ᵈ *prnB*⁻ double mutants by recombination Therefore, *prnA-33*, *prnB-32*, *prnB-35*, and *prnB-36* were selected after NTG mutagenesis of a *yA-2 proA-6 puA-2 prn*ᵈ-20 strain and *prnB-50* was selected after NTG mutagenesis of a strain of genotype *proA-6 puA-2 prn*ᵈ-21 *fwA-1* (*fwA-1* leading to fawn conidial colour) using the method outlined above All of these *prnA*⁻ and *prnB*⁻ mutations are recessive and their locus designations have been confirmed by diploid complementation tests Retention of the *prn*ᵈ mutation has been demonstrated in every case by its recovery on outcrossing

For uptake studies, outcrossed strains carrying *pabaA-1* (*p*-aminobenzoate requirement) were used The *prn*ᵈ-20 *prnB-36* strain also carries *fwA-1* Otherwise, all strains are isogenic, apart from the specified genotype Mycelia were grown for 21 h at 25 °C in appropriately supplemented shaken liquid minimal medium[28] containing either 0 3 or 1% D-glucose as carbon source and either 595 µM uric acid or 10 mM NH₄⁺ (as the (+)-tartrate) as nitrogen source The medium was buffered to pH 6 5 using 10 mmol l⁻¹ orthophosphate (as the sodium salts) Where the medium was made 5 mM with respect to L-proline, this was done after 16 h growth Mycelia were collected by filtration on to nylon mesh washed with sterile, proline-free medium at 25 °C, and resuspended in the appropriate fresh sterile growth medium without proline Aliquots (1 0 ml) of this suspension were transferred to 50 ml Erlenmeyer flasks containing 9 0 ml of the respective growth media without proline L-proline-U-¹⁴C was added to each flask so as to give a final concentration of 200 µM at a specific activity of 125 mCi mol⁻¹ Flasks were incubated at 25 °C in an orbital shaker Duplicate 5 0 ml samples were taken after 0 and 5 min incubation Each sample was filtered on to a preweighed 2 5 cm diameter Whatman GF/A filter paper, washed with 10 ml ice-cold 200 µM L-proline (unlabelled) followed by 10 ml ice-cold distilled water The filters were dried for 3 h at 90 °C, weighed again, placed in 10 ml of toluene scintillant containing 4 0 g 2,5-diphenyloxazole and 40 mg 1,4-bis-(2-(5-phenyloxazoyl))-benzene l⁻¹, and counted in a Packard 3375 Tri-Carb Scintillation Counter Uptake of L-proline was linear up to 20-30 min incubation and was completely inhibited by 5 mM KCN or 100 µg ml⁻¹ 2,4-dinitrophenol Zero time values have been subtracted from 5 min values to correct for nonspecific adsorption Results are expressed in nmol L-proline per min per mg dry weight Corrected values have been obtained by subtracting the activities present in the *prnB-6* strain under each set of growth conditions from the values for the strain specified in the table This correction eliminates the contribution of the minor proline permease (see below)

In addition to the major proline permease whose control is outlined in this paper, there is at least one minor proline permease It is most clearly observed in mutants lacking the major permease (*prnB*⁻) This minor permease, which is responsible for the leakiness of *prnB*⁻ mutants, does not respond to induction by proline but is markedly inhibited or repressed by ammonium This ammonium inhibition or repression is also apparent from the increased sensitivity of proline oxidase to ammonium repression in *prnB*⁻ strains (Table 2) as a result of inducer exclusion Ammonium inhibition or repression of the minor permease is sufficient to account for the slight effect of ammonium on proline transport in the wild type seen above

0 3% glucose was chosen for transport studies in preference to lower concentrations so as not to restrict the energy available for the uptake process itself, even though carbon catabolite repression is still evident at this concentration A strain carrying *creA*ᵈ-1, which relieves carbon catabolite repression of the syntheses of a number of enzymes[11 12], has been included as it also relieves carbon catabolite repression of proline transport

Table 2 Proline oxidase activities of wild type and mutant strains

Relevant genotype	Relative activities of proline oxidase of mycelia grown in media with 595 µM uric acid as N										
	1% D-glucose as C					0 3% D-glucose as C			0 1% D-glucose as C		
	−	5 mM L-proline	5 mM L-proline + 10 mM NH$_4^+$	5 mM L-ornithine	5 mM L-ornithine + 10 mM NH$_4^+$	−	5 mM L-proline	5 mM L-proline + 10 mM NH$_4^+$	−	5 mM L-proline	5 mM L-proline + 10 mM NH$_4^+$
Wild type	4 0	100	27	41	2 0	7 5	130	44	6 0	180	54
prnA-1	0 0	0 0	0 0	0 0	0 0	—	—	—	—	0 0	—
prnB-6	5 0	47	6 0	48	3 0	6 0	96	15	3 0	108	8 0
prn^d-20	6 6	111	63	56	1 7	18	165	88	21	190	86
prn^d-20 prnA-33	0 0	0 0	0 0	0 0	0 0	—	—	—	—	—	—
prn^d-20 prnB-36	1 7	42	12	62	0 0	16	98	3 0	6 0	81	9 0

All strains carry *paba*A-1, and the *prn*^{d}-20 *prn*B-36 strain also carries *fw*A-1 Otherwise, all strains are isogenic, apart from the specified genotype Mycelia for enzyme assays were grown, collected and extracted as described previously[28,29] in appropriately supplemented shaken liquid minimal medium with D-glucose at the specified concentrations as carbon source and 595 µM uric acid as nitrogen source Mycelia were grown for 21 h at 25 °C and, where indicated, 5 mM L-proline or L-ornithine (as the monohydrochloride) with or without 10 mM NH$_4^+$ (as the (+)-tartrate) was added after 16 h growth Cell-free extracts were prepared in 100 mM Tris-HCl buffer *p*H 7 5 containing 500 mM sucrose The crude homogenates were centrifuged at 12,000*g* for 10 min, and the supernatants taken for enzyme assay

Proline oxidase (EC 1 4 3 2) assays were carried out in 1 cm cuvettes in a double beam spectrophotometer, at 30 °C The assay mixtures contained in a total volume of 3 0 ml 200 µmol Tris-HCl *p*H 8 5, 10 µmol 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride, 1 3 µmol N-methylphenazonium methosulphate, 42 nmol FAD, and 0 1 ml enzyme extract containing 200-500 µg protein The reaction was started by adding 150 µmol L-proline The blank cell contained all components of the assay with the exception of proline and the increase in absorbance at 500 nm was measured Soluble protein in extracts was determined by the Biuret method[30], and results were expressed as a percentage of the specific activity of the wild type when grown on 1 % glucose and induced with 5 mM L-proline

Induction of proline oxidase by ornithine requires its conversion to glutamic γ-semialdehyde (or Δ^1-pyrroline-5-carboxylate) as it does not occur in strains lacking ornithine δ-transaminase whereas proline induction occurs normally (data not shown)

proline oxidase, and Δ^1-pyrroline-5-carboxylate (P5C) dehydrogenase in the wild type can be deduced from Tables 1, 2, and 3, respectively All three activities are inducible Proline transport and proline oxidase at least are carbon catabolite-repressible (carbon catabolite repression of P5C dehydrogenase has not yet been investigated) But, whereas proline oxidase and P5C dehydrogenase are ammonium-repressible, proline transport clearly is not

It should be noted that ammonium represses proline oxidase even when there is little or no carbon catabolite repression (at 0 1% glucose) (Table 2) Thus carbon catabolite repression is probably not a prerequisite for nitrogen metabolite repression where the two forms of control coexist Rather, these data suggest that the two forms of control operate more or less independently of each other

Mutants unable to catabolise proline

Selection of *prn*A⁻ and *prn*B⁻ mutations is described in the legend to Table 1 *prn*A⁻ strains lack both proline oxidase and P5C dehydrogenase (Tables 2 and 3) but are normal for proline transport (Table 1) in agreement with their *in vivo* behaviour (Tables 4 and 5) *prn*B⁻ strains are defective in proline transport (Tables 1 and 5) Consistent with this uptake defect, they show somewhat reduced levels of proline oxidase and P5C dehydrogenase when proline, but not ornithine, is used as inducer (Tables 2 and 3)

Regulatory mutations affecting proline catabolism

*are*A^r and *prn*^{d} mutations have been described previously[11] *prn*^{d} mutations are locus-specific suppressors of *are*A^r mutations, uniquely permitting utilisation of proline as nitrogen source

prn^d-20 does not markedly affect uninduced levels of proline uptake, proline oxidase, or P5C dehydrogenase (Tables 1, 2 and 3) It does, however, result in derepression For proline uptake, which is not ammonium-repressible, *prn*^{d}-20 is probably best interpreted as conferring carbon catabolite derepression This is most clear in the presence of a partially carbon catabolite-repressing glucose concentration such as 0 3% (see

Table 3 Δ^1-Pyrroline-5-carboxylate dehydrogenase activities of wild type and mutant strains

Relevant genotype	Relative activities of P5C dehydrogenase of mycelia grown in media with 1% D-glucose as C+5 mM urea as N			
	−	5 mM L-proline	5 mM L-proline + 10 mM NH$_4^+$	5 mM L-ornithine
Wild type I	2 7	100	25	—
Wild type II	0 0	115	20	25
prnA-1	0 0	0 0	—	—
prnB-6	0 0	76	—	—
prn^d-20	0 0	103	56	—

The wild type I, *prn*A-1, *prn*B-6, and *prn*^{d}-20 strains carry *paba*A-1, and the wild type II strain carries *bi*A-1, a biotin auxotrophy, but is *paba*A$^+$ Otherwise, all strains are isogenic, apart from the specified genotype Experimental details are given in the legend to Table 2 with the following modifications and additions Cell-free extracts were prepared in 100 mM Tris-HCl buffer *p*H 7 5 containing 20% (v/v) glycerol Pyrroline-5-carboxylate dehydrogenase (EC number not assigned) assays were carried out in 1 cm cuvettes in a double beam spectrophotometer at 25 °C The assay mixtures, modified after Strecker[31], contained in a total volume of 3 0 ml 250 µmol Tris-HCl *p*H 8 5, 3 0 µmol NAD, and 0 1 ml of enzyme extract The reaction was started by adding 6 µmol DL-Δ^1-pyrroline-5-carboxylic acid, synthesised as described by Strecker[32] The blank cell contained all components except pyrroline-5-carboxylate, and the increase in absorbance was measured at 340 nm Results are expressed as a percentage of the specific activity of wild type I (*paba*A-1) when grown on 1 % glucose and 5 mM urea and induced with 5 mM L-proline

legend to Table 1) From the enzyme data is it not possible to deduce whether derepression of prn^d-20 is the consequence of carbon catabolite derepression or ammonium derepression or both since the relative contributions of the two forms of repression cannot be clearly distinguished (see ref 11) But, by analogy with the permease results, we favour a model in which prn^d-20 relieves carbon catabolite repression of the proline catabolic enzymes Mutations relieving carbon catabolite repression of the enzymes of proline degradation have been reported in *Salmonella typhimurium*[18]

Epistasis relationships

prn^d mutations do not suppress $prnA^-$ or $prnB^-$ mutations (Tables 1, 2, 4, and 5) Therefore prn^d mutations affect the regulation of the proline oxidase and the P5C dehydrogenase which are missing in $prnA^-$ strains and of the proline transport system which is missing in $prnB^-$ strains

both the $prnA$ and $prnB$ genes Diploids of the following (relevant) genotypes were constructed

$$\frac{areA^r\text{-}1\ prn^d\text{-}20\ prnA\text{-}33}{areA^r\text{-}1\ \ +\ \ } \qquad \frac{areA^r\text{-}1\ prn^d\text{-}20\ prnB\text{-}32,}{areA^r\text{-}1\ \ +\ \ }$$

$$\frac{areA^r\text{-}1\ prn^d\text{-}20\ prnB\text{-}35}{areA^r\text{-}1\ \ +\ \ } \qquad \frac{areA^r\text{-}1\ prn^d\text{-}20\ prnB\text{-}36}{areA^r\text{-}1\ \ +\ \ }$$

All are unable to utilise proline as nitrogen source (The presence of prn^d-20 in $areA^r$-1 prn^d-20 $prnA$(or B)$^-$ strains was verified by crossing to an $areA^r$-1 strain and recovering progeny able to grow on proline as nitrogen source (genotype of progeny $areA^r$-1prn^d-20)) Diploids of (relevant) genotype ($areA^r$-1 prn^d-20 +)/($areA^r$-1 + $prnA$-1) and ($areA^r$-1 prn^d-20 +)/($areA^r$-1 + $prnB$-6) utilise proline as well as ($areA^r$-1 prn^d-20)/($areA^r$-1 +) diploids

Table 4 Growth responses of wild type and mutant strains

Relevant genotype	Growth on media containing					
	1% D-glucose as C+ as N at 5 mM			10 mM NH$_4$Cl as N as C+ at 50 mM		
	L-proline	L-ornithine	L-glutamate	L-proline	L-ornithine	L-glutamate
Wild type	++	++	++	+	+	+
$prnA^-$	−	±	++	−	−	+
$prnB^-$	±	++	++	±	+	+
prn^d	++	++	++	+	+	+
$otaA^-$	++	−	++	+	−	+
$areA^r$	−	−	−	+	+	+
$prnA^-\ prnB^-$	−	±	++	−	−	+
$prn^d\ prnA^-$	−	±	++	−	−	+
$prn^d\ prnB^-$	±	++	++	±	+	+
$areA^r\ prn^d$	+	−	−	+	+	+
$prnA^-\ otaA^-$	−	−	++	−	−	+
$areA^r\ prn^d\ prnA^-$	−	−	−	−	−	+
$areA^r\ prn^d\ prnB^-$	−	−	−	±	+	+

Growth tests were carried out at 37 °C on appropriately supplemented solid minimum medium[28] L-ornithine was added as the monohydrochloride, and L-glutamate was added as the monosodium salt The $otaA^-$ allele used here was $otaA$-2, selected as resulting in reduced utilisation of arginine as nitrogen source (L M Palmer and H N Arst, unpublished) It is phenotypically identical to its allele $otaA$-1, leading to loss of ornithine δ-transaminase, selected as preventing endogenously channelled or exogenously supplied arginine and ornithine from supplementing proline auxotrophies resulting from blocks between L-glutamate and L-glutamic γ-semialdehyde (Δ^1-pyrroline-5-carboxylate) in the proline biosynthetic pathway[13,14] The alternative pathway of proline biosynthesis from arginine and ornithine is shown in Fig 1 Gene symbols other than $otaA$ are introduced in the text

One noteworthy relationship is the lack of epistasis of $areA^r$ mutations to $prnB^-$ mutations with respect to supplementation of proline auxotrophies (Table 5) When ammonium is present to repress or inhibit the residual proline uptake present in $prnB^-$ strains (legend to Table 1), $prnB^-$ mutations prevent supplementation of proline auxotrophies by limiting levels of proline $areA^r$ mutations do not have this effect $proA^-\ areA^r$ $prnB^-$ triply mutant strains show an intermediate growth response between that of $proA^-$ (or $proA^-\ areA^r$) and $proA^-$ $prnB^-$ strains (Table 5) This is unlikely to be an allele-specific phenomenon because it occurs with $areA^r$-1, $areA^r$-2, and $areA^r$-19 (formerly designated $amdT$-19[11,19]) Therefore the $areA^r$ phenotype does not include loss of the $prnB$ function under conditions in which it completely abolishes utilisation of proline as a nitrogen source Thus, the lack of ammonium repression of the $prnB$ permease is confirmed *in vivo* by differences in $areA^r$ and $prnB^-$ phenotypes and the lack of epistasis of $areA^r$ to $prnB^-$

Cis-dominance of prn^d-20
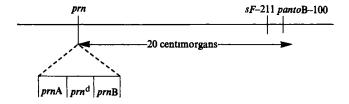
Diploids of (relevant) genotype ($areA^r$-1 prn^d-20)/($areA^r$-1 +) utilise proline as a nitrogen source to an extent which is clearly intermediate between $areA^r$-1 prn^d-20 haploids and $areA^r$-1 prn^+ haploids Cis-trans tests show that the semi-dominance of prn^d-20 reflects the fact that it acts only in cis with respect to

Map positions

Haploidisation analysis[20] located both $prnA$-1 and $prnB$-6 to linkage group VII Meiotic analysis showed them to be tightly linked (2–3 centimorgans) Preliminary work also showed that they are tightly linked to prn^d-20 Therefore crosses containing the following marker configurations were analysed prn^d-20 $prnB$-32 × $prnA$-1, prn^d-20 $prnB$-35 × $prnA$-1, prn^d-20 $prnB$-36 × $prnA$-1, prn^d-20 $prnA$-33 × $prnB$-6, prn^d-21 $prnB$-50 × $prnA$-1 Proline-utilising progeny were isolated and tested for the prn^d phenotype prn^d mutations can be scored in $areA^+$ strains by methods described previously[11] In every case, proline-utilising progeny fell into both prn^d and prn^+ classes For example, among 64 proline-utilising progeny from a cross of configuration prn^d-20 $prnB$-35 × $prnA$-1, there were 27 prn^d-20 and 37 wild type strains Insufficient numbers of progeny were analysed to warrant detailed presentation of quantitative results, but the qualitative results are unequivocal prn^d-20 and -21 map between $prnA^-$ and $prnB^-$ mutations and do not lie markedly closer to one than to the other

As the above crosses were also heterozygous for $pantoB$-100 (pantothenic acid auxotrophy), which maps about 20 centimorgans away, they showed that $pantoB$-100 is located on the $prnB$ side of the prn cluster To orientate the prn cluster on the linkage group VII map, its position with respect to sF-211 (a block beyond sulphite on the sulphate assimilation pathway[21]), closely linked to $pantoB$-100 (ref 22), was deter-

mined by screening for *prn*A-1 among *s*F⁺ *panto*B⁺ progeny of a cross of marker configuration *s*F-211 × *panto*B-100 *prn*A-1 The linkage data can be summarised as follows

*ota*A-2 (ornithine δ-transaminaseless[13]) is also in linkage group VII (L M Palmer, unpublished), but it recombines freely with mutations in the *prn* cluster

*prn*ᵈ Mutations are not allelic to *prn*A— or *prn*B— mutations

There is compelling evidence that *prn*ᵈ mutations cannot be allelic either to *prn*A⁻ or to *prn*B⁻ mutations Firstly, *prn*ᵈ mutations affect both proline uptake and the enzymes proline oxidase and P5C dehydrogenase (Tables 1, 2 and 3) *prn*A⁻ mutations do not affect proline uptake *prn*B⁻ mutations do not affect the levels of the two enzymes (except to an extent which is attributable to reduced inducer uptake when proline is used to induce)

Second, since *prn*ᵈ mutations lack *trans* activity, they do not alter the structure of a diffusible product Since *prn*A⁻ and *prn*B⁻ mutations complement each other, these mutations do alter the structure of diffusible products Given that *cis*-acting regulatory regions are unlikely to overlap with structural genes, *prn*ᵈ mutations cannot be allelic to either *prn*A⁻ or *prn*B⁻ mutations

Gene regulation

We favour a model in which *prn*ᵈ mutations define a distinct regulatory region located between two structural genes, *prn*A and *prn*B Although it is clear that *prn*ᵈ mutations do not occur in the *prn*A and *prn*B genes, we have not yet attempted to show directly that *prn*ᵈ mutations do not alter the structure of the proline permease, proline oxidase, or P5C dehydrogenase Unfortunately, preliminary attempts to obtain conditional *prn*A⁻ and *prn*B⁻ mutations, which might allow definitive identification of structural genes, have been unsuccessful (D J Cove and H N Arst, unpublished) There is no evidence that mutations in genes other than *prn*A can lead to loss of proline oxidase, but very recently a mutation whose

in vivo behaviour suggests that it eliminates P5C dehydrogenase has been obtained (H N Arst, unpublished) It has not yet been located

A divergent orientation with an internal control region is an obvious way to organise two clustered genes (or sets of genes) having at least one control element in common and one control element not in common Whereas both the *prn*A and the *prn*B products are probably subject to induction and carbon catabolite repression, only the *prn*A product is likely to be subject to nitrogen metabolite repression As *prn*ᵈ mutations affect the control of both *prn*A and *prn*B but do not significantly affect induction, they would seem to involve principally carbon catabolite derepression Future work will be directed towards elucidating the induction process and, in the case of *prn*A, the mechanism of nitrogen metabolite repression

An intriguing aspect of *prn*ᵈ mutations is the high frequency with which they are induced (H N Arst, unpublished), suggesting that the target in the region between *prn*A and *prn*B where mutation can result in the *prn*ᵈ phenotype is rather large Perhaps *prn*ᵈ mutations occur in a receptor site for a negative control element There are other indications that carbon catabolite repression might be mediated by a negative control mechanism in *A nidulans* *cre*Aᵈ-1, leading to carbon catabolite derepression, is recessive[12], and *cre*Aᵈ mutations are not uncommon (C Bailey and H N Arst, unpublished) The role of the *cre*A gene in carbon catabolite repression has yet to be established, but these observations are consistent with its coding for a repressor which binds to sites such as that where *prn*ᵈ mutations occur Extensive attempts to obtain mutants simultaneously defective in the utilisation of several carbon sources have failed (C Bailey and H N Arst, unpublished), which is consistent with the expected rarity of a super-repressed phenotype Moreover, a negative model for carbon catabolite repression provides a rationale for the observation[11] that, among a number of nitrogen sources on which revertants of *are*Aʳ strains have been obtained, specific suppressor mutations have been selected only for proline and acetamide These are the only two nitrogen sources whose utilisation by *are*Aʳ mutants can be achieved by the alleviation of carbon catabolite repression Obtaining *cis*-acting regulatory mutations adjacent to *amd*S, the structural gene for acetamidase[23], is complicated by the occurrence of mutations in two unlinked regulatory genes, *amd*R[11,24] and *amd*A (H N Arst, unpublished), suppressing *are*Aʳ mutations on acetamide While revising this manuscript, we learned that Hynes[25] obtained a *cis*-dominant regulatory mutation tightly linked to *amd*S He prefers to interpret his mutation as leading to increased inducibility, but his data do not eliminate the possibility that it leads to carbon catabolite derepression

Table 5 Effects of various mutations on supplementation of proline auxotrophies

Relevant genotype	Growth using supplementation by		
	5 mM L-proline	250 μM L-proline	5 mM L-ornithine or L-arginine
*pro*A⁻	++	+	++
*pro*A⁻ *prn*A⁻	++	+	++
*pro*A⁻ *prn*B⁻	++	−	++
*pro*A⁻ *prn*ᵈ	++	+	++
*pro*A⁻ *ota*A⁻	++	+	++
*pro*A⁻ *are*Aʳ	++	+	−
*pro*A⁻ *are*Aʳ *prn*B⁻	++	±	++
*pro*A⁻ *prn*ᵈ *prn*B⁻	++	−	++
*pro*A⁻ *prn*A⁻ *prn*B⁻	++	−	++
*pro*A⁻ *are*Aʳ *prn*ᵈ *prn*B⁻	++	±	++
*pro*B⁻	++	+	++
*pro*B⁻ *prn*B⁻	++	−	++
*pro*B⁻ *ota*A⁻	++	+	−

Growth tests were carried out at 37 °C on appropriately supplemented solid minimal medium[28] with 1% D-glucose and 10 mm NH₄⁺ (as the (+)-tartrate) as C and N sources, respectively *pro*A-6 and *pro*B-3 were utilised as the *pro*A⁻ and *pro*B⁻ alleles respectively They are closely linked but non allelic mutations to proline auxotrophy and are blocked between L-glutamate and L-glutamic γ-semialdehyde (Δ¹-pyrroline-5-carboxylate) on the proline biosynthetic pathway[14] Gene symbols other than *pro*A and *pro*B are introduced in the text and the legend to Table 4

Note that *pro*A⁻ *are*Aʳ *prn*B⁻ triple mutants respond better to limiting proline levels (250 μM) than *pro*A⁻ *prn*B⁻ double mutants This is probably the result of the lack of an ammonium-repressible ammonium transport system in *are*Aʳ strains[11], assuming that ammonium must enter the cell to inhibit or repress residual proline uptake

Whatever the mechanism of carbon catabolite repression in *A nidulans*, the nature of the effector involved is an open question Neither cyclic AMP nor its N^6-2'-O-dibutyryl derivative seem to reverse carbon catabolite repression in any of several sensitive *in vivo* diagnostic systems (C Bailey and H N Arst, unpublished) But there is no evidence that these compounds actually enter the cell, for they neither supplement adenine auxotrophies nor serve as nitrogen sources

We believe this to be the first definitive demonstration of an operon-type structure (as opposed to simple tight linkage of functionally related genes) in an eukaryotic organism

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Note added in proof Recessive mutations eliminating P5C dehydrogenase whilst not reducing levels of proline transport or proline oxidase define another gene, *prnC prnC⁻* mutations are tightly linked to the other *prn* mutations, with the map, order being *prnA prnB prnC* *pantoB*

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Initiator constitutive mutation with an 'up-promoter' effect in *Aspergillus nidulans*

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A mutation leading to strong constitutivity for a uric acid-xanthine permease in the lower eukaryote Aspergillus nidulans has been found to be tightly linked to the putative structural gene whose expression it controls in the cis configuration In addition, it increases the maximal induced level of transport 2 5-fold This is the first demonstration of an initiator or promoter mutation in an eukaryote

STUDIES on the regulation of enzyme synthesis in the fungus *Aspergillus nidulans* are increasingly uncovering instances in which such synthesis is subject to multiple forms of regulation For example, in the accompanying paper[1], it is shown that the synthesis of enzymes involved in proline degradation is inducible and is also repressible by both carbon catabolite and nitrogen metabolite repression Another example is provided by the *hxB* gene, which codes for a subunit common to two enzymes[2], each of which is subject both to a specific induction system and to nitrogen metabolite repression[3-5] It is reasonable to assume that adjacent to each multiply regulated structural gene there are receptor sites specific for the respective proteins coded by each regulatory gene involved These sites may or may not overlap Evidence for multiple regulatory sites has been reported in the *lac*, *ara*, and, notably, *gal* operons of *Escherichia coli* (refs 6, 7, 8, and references therein)

In *Aspergillus nidulans*, a permease specific for uric acid and xanthine[9] is both inducible and ammonium-repressible Expression of the gene coding for the permease requires the products of two positive control genes the *uaY* gene, necessary for induction (refs 3, 10, and D J Cove, H N Arst, and C

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Scazzocchio, unpublished), and the *areA* gene, necessary for the expression of all genes subject to nitrogen metabolite (ammonium) repression[5] Presumably the structural gene (or its RNA transcript) for the uric acid–xanthine permease is adjacent to two receptor sites, one able to bind the product of the *uaY* gene in the presence of coinducer (uric acid[10]) and another able to bind the product of the *areA* gene in the absence of corepressor (presumably ammonium) As a great number of enzymes and permeases are subject to ammonium repression, regions of (presumably) DNA able to bind the *areA* product should be widely distributed throughout the genome Since activities coded by different genes respond in different ways to almost every allele of the *areA* gene[5], one can only presume that these recognition sites are not all identical Moreover, mutations in the *areA* gene can also affect the maximal level of synthesis of any of these enzymes or permeases[5], suggesting that at least a functional overlap might exist between the receptor site for the *areA* product and a promoter site In the *lac* operon of *E coli*, not only is there at least a functional overlap between the promoter site (for binding RNA polymerase) and the binding site for the catabolite activator protein[11,12], but most operator constitutive mutations show more or less marked promoter effects[13] Recent research in the bacteriophage λ has shown a physical overlap of promoter and operator sites[14,15]

A general method to identify control regions adjacent to structural genes under *areA* control should be to select *cis*-acting mutations which permit the expression of a given structural gene in the presence of a non functional *areA* allele But most *areA* mutations result in loss of many, if not all, of the activities subject to ammonium repression As the genes coding for enzymes catalysing sequential steps in a given pathway are generally not clustered in *Aspergillus*[16], mutations at more than one receptor site would be necessary to

Table 1 *In vivo* responses to xanthine, uric acid, and their analogues

Relevant genotype	Inhibition of hypoxanthine utilisation by allopurinol at		Sensitivity to Inhibition of green pigment formation by 2-thioxanthine or 2-thiouric acid at		Inhibition of growth of strains also carrying an *al*X—mutation by xanthine or uric acid at	
	500 ng ml⁻¹	20 ng ml⁻¹	100 μg ml⁻¹	10 μg ml⁻¹	1 mg ml⁻¹	50 μg ml⁻¹
Wild type	S	R	S	R	S	R
*uap*A⁻	R	R	R	R	R	R
uap-100	S	S	S	S	S	S
uap-100 *uap*A⁻	R	R	R	R	R	R

S=Sensitive, R=resistant

Five mutations leading to defective transport of uric acid and xanthine (*uap*A⁻)[9] were obtained, after NTG mutagenesis, in a strain of genotype *paba*A-1, as conferring resistance to 100 μg ml⁻¹ 2-thioxanthine, a xanthine analogue[9], on hypoxanthine as sole nitrogen source The use of hypoxanthine discards all mutants lacking xanthine dehydrogenase[24], leaving *uap*A⁻ mutants as the major class One mutation, *uap*A-24, was used for transport and genetical studies. Because of the tight linkage of *uap*A⁻ mutations to *uap*-100 and the lack of external markers, it was not feasible to obtain *uap*-100 *uap*A⁻ double mutants by recombination Therefore other *uap*A⁻ mutations were selected, using the above method, in a strain carrying *uap*-100 (*uap*-100 *pyro* A-4, auxotrophic for pyridoxine) One mutation, *uap*A-37, was selected for further work and was shown not to complement with *uap*A-24 in diploids *uap*A-24 and *uap*A-37 are both recessive and tightly linked (< 1 centimorgan) to *uap*-100 and are therefore allelic These mutations have been located[25] in linkage group I Growth tests were made on appropriately supplemented solid minimal medium[26] at 37 °C Inhibition by allopurinol was tested using 100 μg ml⁻¹ hypoxanthine as nitrogen source Inhibition by 2-thioxanthine and 2-thiouric acid was tested used 3 3 mH NH₄⁺ as nitrogen source Inhibition by xanthine and uric acid was tested using 10 mM NO₃⁻ as nitrogen source Allopurinol specifically inhibits xanthine dehydrogenase and thus the utilisation of hypoxanthine as nitrogen source[3] 2-thiouric acid inhibits the conversion of yellow to green conidial pigment[9,24,27] 2-thioxanthine is converted by xanthine dehydrogenase to 2-thiouric acid[9,24] Xanthine and uric acid are toxic to strains lacking allantoinase (*al*X⁻) resulting from allantoin accumulation[9] Allopurinol, 2-thiouric acid, 2-thioxanthine, xanthine and uric acid are all substrates of the *uap*A permease[3,9]

obtain a revertant As even single mutations resulting in the ability to bypass the *are*A product or to accommodate a modified *are*A product should be rare, a requirement for multiple mutational events would make the method impracticable

Nevertheless, advantage can be taken of the wide variety of phenotypes displayed by *are*A mutations In particular, one allele, *are*A-102 (formerly designated *amd*T-102), was selected as resulting in derepression of acetamidase[17] But, *are*A-102 also leads to reduced levels of formamidase[17,18] and notably to an almost total lack of transport activity for uric acid and xanthine, whilst not affecting the catabolism of these compounds once inside the cell[5] Thus, a revertant able to grow on uric acid and xanthine as nitrogen sources should result from a single mutational event in a *cis*-acting element controlling the uric acid–xanthine permease This paper describes one such *cis*-dominant mutation, designated *uap*-100, which results in strong constitutivity of the permease as well as a marked 'up-promoter' effect (An 'up-promoter' effect is an increase in the maximal level of expression of the adjacent structural gene) *uap*-100 does not, however, confer derepression *vis-à-vis* ammonium This last characteristic is somewhat surprising in view of the method of selection and will be discussed below

Selection of *uap*-100

uap-100 was obtained after N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis[19] of a (*p*-aminobenzoate-requiring) *are*A-102 strain (*paba*A-1 *are*A-102) Conidiospores

were plated on medium containing uric acid as sole nitrogen source, and 42 revertants were isolated Forty-one of these revertants have lost at least one other of the characteristics of the *are*A-102 phenotype and presumably contain second-site reversions in the *are*A gene One revertant has exclusively regained the ability to utilise uric acid and xanthine whilst retaining all other characteristics associated with the *are*A-102 mutation As predicted, it carries a specific suppressor mutation Selection of *uap*A⁻ mutations is described in the legend to Table 1

In vivo properties of *uap*A⁻ and *uap*-100 mutations

Table 1 compares *uap*A⁻, *uap*-100 and wild type strains with regard to sensitivity to a number of purines and purine analogues taken up by way of the *uap*A permease[3,9] Whereas *uap*A⁻ mutations confer resistance to these compounds, *uap*-100 leads to marked hypersensitivity *uap*A⁻ mutations are completely epistatic to *uap*-100

Transport and enzyme studies

2-thiouric acid is a gratuitous inducer of both xanthine dehydrogenase I and urate oxidase in *A nidulans*[20] Tables 2 and 3 show that it also induces a uric acid permease which is missing in strains carrying *uap*A-24 The residual uric acid transport in these strains occurs by means of permeases specified by other genes (H N Arst and C Scazzocchio, unpublished)

Table 2 Induction of uric acid transport activity

Relevant genotype	¹⁴C/³H 2-thiouric acid present in growth medium at (μM)			
	0	2 77	27 7	277
uap⁺	0 0431	0 2763	0 2065	0 1265
uap-100	0 4200	0 6743	0 5403	0 3399

The method for uptake studies has been described in detail previously[28] The ¹⁴C counts monitor uric acid uptake while the ³H counts serve as a measure of protein synthesis and hence growth In addition to the *uap* genotype specified above, both strains carry *y*A-2 (yellow conidial colour), *lu*A-1 (L-leucine auxotrophy), *hx*B-13 (loss of xanthine dehydrogenase), and *ua*Z-11 (loss of urate oxidase) but are otherwise isogenic The use of urate oxidaseless strains allows uptake measurements to be made in the absence of any uric acid catabolism[29] But *ua*Z⁻ strains accumulate uric acid and are therefore pseudo-constitutive for uric acid-induced activities unless they also lack xanthine dehydrogenase[20,30] Hence it is essential to use xanthine dehydrogenaseless strains to study induction Mycelia were grown for 21 h at 37 °C on solid (growth) medium[26] supplemented with 10 μg l⁻¹ biotin, 5 mM urea, 1 mM 4,5-³H-L-leucine at a final specific activity of 500 mCi mol⁻¹, and 2-thiouric acid at the specified concentrations Mycelia were incubated for 30 min at 37 °C in uptake medium supplemented with 10 μg l⁻¹ biotin, 5 mM urea, 1 mM L-leucine (unlabelled), and 59 5 μM 2-¹⁴C-uric acid at a final specific activity of 3 36 Ci mol⁻¹

Table 3 Ammonium repression of uric acid transport activity

Relevant genotype or calculation	None	¹⁴C/³H Additions to growth medium 27 7 µM 2-thiouric acid	10 mM NH₄⁺	27 7 µM 2-thiouric acid +10 mM NH₄⁺
*uap*A-24	0 0201	0 0232	0 0120	0 0162
uap⁺	0 0436	0 2206	0 0470	0 0542
uap-100	0 3931	0 4645	0 0906	0 1165
Activity ratio	15 9	2 3	2 2	2 1

The experimental method was the same as that in the legend to Table 2, except that the additions to the growth medium were those specified above All strains carry *y*A-2, *lu*A-1, *hx*B-13, and *ua*Z-11 (as in Table 2) in addition to the *uap* genotype given above but are otherwise isogenic The activity ratio is calculated as (¹⁴C/³H for *uap*-100 — ¹⁴C/³H for *uap*A-24)/(¹⁴C/³H for *uap*⁺ — ¹⁴C/³H for *uap*A-24) for each set of growth conditions Since the residual uric acid transport activity in *uap*A-24 is probably attributable to permeases other than that specified by the *uap*A gene (H N Arst and C Scazzocchio, unpublished), the activity ratio expresses the ratio of uric acid transport activity by way of the *uap*A permease in the *uap*-100 strain to that in the wild type for any given set of growth conditions

The decline in uptake as higher concentrations of thiouric acid are used to induce probably reflects inhibition of uric acid uptake by preloaded 2-thiouric acid Tables 2 and 3 show that strains carrying *uap*-100 are strongly constitutive for uric acid uptake That the permease responsible for this uptake is the one missing in *uap*A⁻ mutants is shown by the complete epistasis of *uap*A⁻ mutations to *uap*-100 (Table 1) On maximal induction, strains carrying *uap*-100 have 2 5 times the level of uric acid transport found in the induced wild type (Tables 2 and 3) Both induced wild type and induced *uap*-100 show approximately fourfold repressibility by ammonium (Table 3)

Table 4 Induction of urate oxidase by a gratuitous inducer

Concentration of 2-thiouric acid		Urate oxidase specific activity Wild type	*uap*-100
0		<1	<1
27 7	pM	<1	<1
134	pM	3 0	20 9
277	pM	29 3	40 2
2 77	nM	59 5	49 2
27 7	nM	57 6	56 5
277	nM	59 3	50 1

Both strains carry a biotin auxotrophy (*bi*A-1) and are, apart from the specified genotypes, isogenic Mycelium was grown for 20 h at 25 °C in shaken liquid minimal medium[26] supplemented with 10 µg l⁻¹ biotin and containing 5 mM urea 2-thiouric acid was added after 15 h growth Preparation of cell-free extracts and urate oxidase (EC 1 7 3 3) determinations have been described previously[29] Specific activities are defined as nM substrate transformed per min × mg soluble protein Soluble protein in extracts was determined by the Biuret method[31]

Data in Table 4 show that the enhancement of uric acid uptake activity by *uap*-100 is reflected in enhanced sensitivity of urate oxidase induction to 2-thiouric acid The difference is most striking at 134 pM 2-thiouric acid where the *uap*-100 strain is induced to 37% of its maximal level while the wild type is induced to only 5% of its maximal level Comparable

results were also obtained for xanthine dehydrogenase I (data not shown)

Data in Tables 4 and 5 show that uninduced and induced levels of xanthine dehydrogenase I and urate oxidase are not affected by the *uap*-100 mutation These enzymes are, as is the *uap*A permease, under the positive control of the *ua*Y gene (refs 3, 10, and D J Cove, H N Arst and C Scazzocchio, unpublished) Data in Table 5 also show that the apparent degree of ammonium-repressibility of the two enzymes is reduced in *uap*-100 strains when uric acid is used as the inducer As *uap*-100 has no such effect when hypoxanthine is used to induce, part of the apparent ammonium-repressibility can be attributed to inducer exclusion Hypoxanthine, which enters the cell by another permease[9], is able to induce only because it is converted intracellularly to uric acid[20]

Cis-dominance of *uap*-100

Table 6 shows the apparent dominance of *uap*-100 in diploids homozygous for *are*A-102 In this case, the *in vivo* test does not distinguish between semidominance and complete dominance In any event, Table 6 shows clearly that *uap*-100 only affects the expression of a *uap*A gene when located *cis* to it The presence of *uap*-100 in *uap*-100 *uap*aA-37 *are*A-102 strains was verified by crossing to an *are*A-102 strain and recovering progeny able to grow on uric acid as nitrogen source (genotype of progeny *uap*-100 *are*A-102) The presence of *uap*A-37 or *uap*A-24 in strains also carrying *are*A-102 was confirmed by outcrossing to wild type and recovering approximately 25% *uap*A⁻ *are*A⁺ progeny

Nature of the *uap*-100 mutation

We have not yet established that *uap*A is the structural gene for the uric acid–xanthine permease The selection of mutants with altered substrate specificity, now in progress, might provide the necessary proof

Three hypotheses could account for the properties of *uap*-100 (1) The 'up-promoter' effect of *uap*-100 might reflect an altered structure of the permease, increasing the efficiency of uptake The strong constitutivity of *uap*-100 virtually eliminates this

Table 5 Inducer exclusion by ammonium

Addition at 15 h or calculation	Specific activities Xanthine dehydrogenase Wild type	*uap*-100	Urate oxidase Wild type	*uap*-100
None	10 8	5 8	1 7	1 8
Hypoxanthine	43 9	33 3	35 7	24 1
Hypoxanthine+ammonium	20 6	15 8	3 8	3 0
% Repression with hypoxanthine as inducer	53 1	52 5	95 8	87 6
Uric acid	55 0	41 6	29 8	17 1
Uric acid+ammonium	21 1	27 7	1 5	8 1
% Repression with uric acid as inducer	61 7	33 4	94 1	72 8

Experimental details are given in the legend to Table 4 with the following modifications and additions 50 µg ml⁻¹ hypoxanthine or uric acid with or without 10 mM ammonium (as the (+)-tartrate) was added after 15 h growth Xanthine dehydrogenase I (EC 1 2 1 37) was determined according to Scazzocchio *et al*[3] % Repression=(1-(ammonium-repressed specific activity)/(non-repressed (induced) specific activity))×100

Table 6 *Cis*-dominance of *uap*-100

Relevant genotype			Utilisation of (as nitrogen source)		
			Hypoxanthine	Xanthine	Uric acid
Wild type			−	+	+
*are*A-102			+	−	−
uap-100 *are*A-102			+	+	+
$\dfrac{uap\text{-}100}{+}$ $\dfrac{areA\text{-}102}{areA\text{-}102}$			+	+	+
$\dfrac{uap\text{-}100}{+}$ $\dfrac{uapA\text{-}37}{+}$ $\dfrac{areA\text{-}102}{areA\text{-}102}$			+	−	−
$\dfrac{uap\text{-}100}{+}$ $\dfrac{+}{uapA\text{-}24}$ $\dfrac{areA\text{-}102}{areA\text{-}102}$			+	+	+

Two independent diploids were constructed for both *cis* and *trans* configuration dominance tests Growth tests were carried out at 37 °C on solid minimal medium[26] with purines added at 100 μg ml⁻¹

possibility (2) *uap*-100 is a constitutive allele and *uap*A⁻ mutations are noninducible alleles in a positive control gene This is extremely unlikely as *uap*-100 and *uap*A⁻ mutations do not affect the regulation of the enzymes induced together with the uric acid permease Moreover, the strong 'up-promoter' effect would be unprecedented and difficult to accommodate to this model (3) *uap*-100 is a mutation in a control region adjacent to a structural gene, *uap*A, coding for the uric acid–xanthine permease This hypothesis is consistent with all the data and is definitely the most attractive

Since the *uap*A gene is under the control of two positive regulatory genes, *ua*Y and *are*A, *uap*-100 can be described as an initiator constitutive mutation Work is in progress to establish the nature of the interaction between *uap*-100 and the *ua*Y and *are*A products Preliminary *in vivo* evidence indicates that *uap*-100 partially suppresses *ua*Y⁻ mutations for permeation but does not alleviate the stringent requirement for the *are*A product This latter finding is in essential agreement with the lack of effect of *uap*-100 on the ammonium-repressibility of the *uap*A permease Therefore the suppression of *are*A-102 by *uap*-100 implies that *uap*-100, in contrast to its wild type allele, can accommodate to the modified *are*A product present in *are*A-102 strains Probably the 'up-promoter' effect of *uap*-100 contributes to the magnitude of the suppression 'Up-' and 'down-promoter' effects are a feature of many initiator constitutive mutations in the *ara* (ref 21) and *mal* (ref 22) systems of *E coli*

In addition to the *uap*A permease, *are*A-102 results in the loss of at least one other permease for uric acid and xanthine (H N Arst and C Scazzocchio, unpublished) Although *are*A-102 leads to somewhat reduced levels of a number of enzymes and permeases[5, 17, 18, 23] these two (or more) permeases with overlapping specificities are the only activities which seem to be totally absent in *are*A-102 strains This may be the first direct clue that similarity of binding sites for the *are*A product constitutes a physiologically significant form of control The possibility that *are*A binding sites might exist in discrete classes related to each other by changes involving only a few bases is

suggested by the observation (H N Arst and C Scazzocchio, unpublished) that intracistronic reversions in an *are*A-102 strain yield new *are*A alleles with a phenotype mirroring that of *are*A-102 They result in recovery of uric acid and xanthine uptake activities but in loss of acetamidase

Pairs of *are*A alleles with antisymmetrical properties and their respective initiator specific suppressors could well provide the means to identify and classify initiator sites in an eukaryote by purely genetical means

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Determinant of cistron specificity in bacterial ribosomes

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The sequence of the 3'-terminus of 16S RNA from different bacteria has been determined Complementarity relationships between this sequence and a purine-rich tract in the ribosome binding site of different bacterial mRNAs suggest that the 3'-end of 16S RNA determines the intrinsic capacity of ribosomes to translate a particular cistron

INITIATION of protein synthesis in bacteria involves the specific binding of the small ribosome subunit to a region of the mRNA containing the initiation codon As the initiating 30S subunit discriminates against the many internal AUG or GUG codons and selects only the AUG or GUG triplet at the beginning of a cistron, some feature of the mRNA other than the presence of AUG must be necessary for ribosome recogni-

Table 1 Determination of the 3'-sequence of *C crescentus* 16S RNA

Number of stepwise degradations before labelling with ³H-isoniazid	Radioactivity in nucleoside hydrazones released by pancreatic ribonuclease*				Sequence
	G-iNzd	U-iNzd	A-iNzd	C-iNzd	
0	3 5	83 8	8 1	4 6	PyU_{OH}
1	3 5	8 7	8 0	79 9	$PyCU_{OH}$
2	5 6	74 3	6 4	13 7	$PyUCU_{OH}$
3	8 3	70 4	9 9	11 3	$PyUUCU_{OH}$
4	6 0	71 8	5 1	17 1	$PyUUUCU_{OH}$
5	6 4	19 2	7 3	67 1	$PyCUUUCU_{OH}$
6	7 6	13 3	8 0	71 1	$PyCCUUUCU_{OH}$
7	8 6	62 9	11 4	17 1	$PyUCCUUUCU_{OH}$

C crescentus (ATCC 15252) was grown and collected as described by Leffler and Szer[2] RNA was extracted directly from the cell pellet with a mixture of phenol/cresol-aminosalicylate[35] DNA, low molecular weight RNA and polysaccharide were removed by washing with 3 M sodium acetate[35] 16S RNA was isolated on 5–20% (w/v) sucrose gradients After 3'-terminal labelling, 16S RNA was digested with pancreatic ribonuclease (100 μg per mg RNA) at 20° C for 4 h in 0 01 M Na/K phosphate buffer (*p*H 7 4) A sample of the digest (10 or 20 μl) was mixed with 20 μl of unlabelled mononucleoside hydrazones[39] and electrophoresed on Whatman 3MM paper at 40 V cm⁻¹ for 2 h in 0 1 M sodium formate (*p*H 3)
*Expressed as a percentage of total radioactivity in nucleoside hydrazones separated by paper electrophoresis

tion Such recognition probably involves some as yet undefined component or feature of the untranslated sequence on the 5'-side of the initiator codon After the specific interaction of mRNA with the 30S subunit, stabilisation of the initiation complex is dependent on initiation factors[1-3], and possibly on the 'fractional' ribosomal protein S_1 (ref 4)

Cistron specificity

A number of reports demonstrate that ribosomes from different bacterial species show cistron specificity in the translation of natural mRNAs *in vitro*, irrespective of the source of the initiation factors used[2,5-11] Thus, ribosomes from *Micrococcus cryophilus* or *Pseudomonas* translate protein from all three cistrons of MS2 RNA in the same relative amounts as those from *Escherichia coli*[8] Under comparable conditions *Bacillus stearothermophilus* ribosomes translate only the A-protein cistron of R17 or f2 RNA (refs 5–7) *Caulobacter crescentus* ribosomes are unable to translate any of the MS2 RNA cistrons[2], correspondingly, *E coli* ribosomes do not translate RNA from the *C crescentus* phage Cb5 (ref 2) A similar cistron selectivity is also demonstrated, in the absence of initiation factors, in a weak, but species-specific interaction of ribosomes with certain phage RNAs (ref 4)

Studies with hybrid ribosomes show that the specificity of ribosome binding and translation of natural mRNAs is primarily a function of the 30S subunit[2,7] Two recent reports aimed at determining the component of the 30S subunit responsible, argue that both a ribosomal protein (particularly protein S12) and the 16S RNA determine the specificity of initiation[12,13] The type of recognition process involved, however, is unknown

Base sequence or secondary structure?

The recognition of mRNA initiation signals by 30S subunits could conceivably be determined by a specific base sequence on the 5'-side of the initiation triplet, by the degree or type of secondary structure in this region of the mRNA, or by both factors Ribonuclease digestion of initiation complexes formed on specific mRNAs has made it possible to determine the base sequence of ribosome-binding sites for several mRNA cistrons[6,14-23] Although some of these ribosome-protected sequences probably have a fairly high degree of secondary structure[6,14,19,24], no such secondary structure seems possible for other initiation sites[23,25,26] It therefore seems unlikely that the 30S subunit specifically recognises some feature of the secondary structure of the ribosome-binding site sequence, particularly since a reduction in structure on partial denaturation of intact mRNAs generally increases, rather than decreases, the number of available initiating sites[27,28] This observation therefore suggests that the specific binding of

ribosomes to mRNA may depend on the ribosome-binding site being in an open, single-stranded form, rather than in a structured conformation

It is significant therefore that all coliphage RNA ribosome-binding sites examined to date contain all or part of the purine-rich sequence 5'-AGGAGGU-3' in a similar relative position on the 5'-side of the initiator triplet AUG The ribosome binding site of an endogenous *E coli* mRNA also contains part of this sequence, AGGA (ref 23) We have previously shown that the 3'-terminus of *E coli* 16S RNA contains the sequence 5'-ACCUCCU-3' which is the complement of AGGAGGU (ref 29) This, together with evidence that an intact 3'-terminus of 16S RNA is necessary for protein synthesis in bacteria[30-32] and more recent data suggesting a specific role for the 3'-terminus in initiation[33], have prompted us to suggest that the 3'-terminal sequence of 16S RNA may have a direct base-pairing role in the initiation of protein synthesis on natural mRNAs (ref 29)

This hypothesis predicts that there will be a positive correlation between the translation of a particular bacterial mRNA cistron by bacterial ribosomes, and the degree of complementarity which exists between the ribosome binding site sequence of that cistron and the 3'-terminal sequence of the 16S ribosomal RNA We have therefore determined the 3'-terminal sequences of 16S RNA from *P aeruginosa*, *B stearothermophilus* and *C crescentus* since information exists on both the capacity of ribosomes from these bacteria to translate various bacterial mRNA cistrons, and on the ribosome binding site sequences of the cistrons concerned

3'-terminal sequences

A procedure for the stepwise removal of seven to eight nucleotides from the 3'-terminus was applied to 16S RNA isolated from bacteria (see Tables 1 and 2) This is a cyclic process and involved removal of the periodate-oxidised 3'-nucleoside by incubation in 0 33 M aniline, digestion with alkaline phosphatase to release the 3'-phosphate and periodate-oxidation of the resultant 2',3'-hydroxyl groups[29,34] After removal of successive 3'-nucleosides, the RNA was labelled by condensation with ³H-isoniazid[35] and digested with specific nucleases The labelled 3'-terminus was characterised by chromatography of the ribonuclease digest on DEAE-Sephadex and identification of any 3'-nucleoside hydrazones by paper electrophoresis[36,37]

An outline of the sequence determination for *C crescentus* 16S RNA is shown in Table 1 The 3'-sequences of 16S RNA from the other bacteria, determined by the same method, are listed in Table 2 A general feature of all the 3'-termini is the high proportion of pyrimidine residues Apart from the terminal adenosine, the seven terminal nucleotides at least, are invariably uridylic acid or cytidylic acid

Complementarity to coliphage ribosome-binding site sequences

We have previously shown that the 3'-terminal nucleotides of *E coli* 16S RNA could form seven, four and three base pairs with the appropriate region of the R17 A-protein, replicase and coat-protein ribosome-binding sites, respectively (see Table 3 and ref 29) The extent of this pairing relates closely to the ribosome-binding capacities of the three isolated initiator regions of R17 RNA since *E coli* ribosomes discriminate in favour of the A-protein initiator fragment some fortyfold and elevenfold over the coat and replicase sites, respectively[38] This is in contrast to initiation of the three cistrons on intact R17 RNA where some 20 moles of coat protein and 5 moles of replicase are synthesised for each mole of A-protein[44] Under these conditions the secondary structure of the intact phage RNA presumably impedes binding of the 30S subunit to the A-protein and replicase initiator regions[38]

The 3'-end of *Ps aeruginosa* 16S RNA is identical to that of *E coli* for seven nucleotides, it can be aligned such that five, four and three to four base pairs are possible with the ribosome-binding site sequences of the A-protein, replicase and coat-protein cistrons of R17 RNA, respectively (Table 3) It has been previously shown[8] that *Pseudomonas* ribosomes translate all three cistrons of MS2 RNA in a manner analogous to that of *E coli* ribosomes

B stearothermophilus (and *B subtilis*) 16S RNA has a 3'-sequence significantly different to that of *E coli* and *Ps aeruginosa* 16S RNA (Table 2) Some complementarity ($\geqslant$ four base pairs) exists, however, between this sequence and the ribosome-binding sites of both the A-protein and replicase cistrons of R17 RNA (Table 3) The degree of complementarity possible with the coat-protein binding site is considerably less (one to two base pairs) *B stearothermophilus* ribosomes bind significantly only to the A-protein site on native f2 or R17 RNA (refs 6 and 7) Appreciable translation by *B stearothermophilus* ribosomes of both the replicase and A-protein cistrons does occur, however, after unfolding of the RNA with formaldehyde[28], under these conditions no coat protein is synthesised *B stearothermophilus* ribosomes also exhibit a relatively high level of recognition of the R17 replicase initiation site when assayed at 49 °C (ref 13), the T_m of the short helix containing the R17 replicase initiator region is 48 °C in 0 05 M Na⁺ (ref 24) *B stearothermophilus* ribosomes are therefore intrinsically able to recognise the A-protein and replicase sites but not the coat-protein site, as would be predicted if a minimum of three to four base pairs were necessary to permit stable interaction Presumably the conformation of the native, intact phage RNA usually renders the replicase site inaccessible to *B stearothermophilus* ribosomes

B stearothermophilus ribosomes bind to only a single site on Qβ RNA at 65 °C, which does not correspond to any of the three normal initiator regions[26] The ribonuclease-protected fragment is similar in size to that bound by ribosomes during productive initiation on the A-protein cistron of R17 RNA It contains no initiation triplet, showing that mRNA can be specifically recognised and bound by ribosomes independently of polypeptide chain initiation[26] The fragment, however, contains a polypurine tract of composition G(AAAG,AG,G,G, G)A in a similar relative position to that of AGGAGGU in the R17 A-protein site[26] It is significant therefore that the complement of the 3'-terminus of *B stearothermophilus* 16S RNA is AGAAAGGA

As the 3'-sequence of *C crescentus* 16S RNA is similar to that of *B stearothermophilus* 16S RNA (Table 2), one could predict a similar recognition by *C crescentus* ribosomes of both the A-protein and replicase cistrons of R17 RNA (Table 3) This prediction is not inconsistent with the observed failure of *Caulobacter* ribosomes to translate MS2 RNA at 37 °C, as under these conditions the initiation sites for the A-protein and replicase cistrons are presumably largely masked by the secondary structure of the phage RNA (refs 24 and 38) This is analogous to the very low level of translation of f2 RNA by *B stearothermophilus* ribosomes at 37 °C compared to that found at 65 °C or after mild denaturation of the RNA with formaldehyde[28] It is significant that considerable translation of formaldehyde-treated MS2 RNA occurs with *Caulobacter* ribosomes[2], although the identity of the translated cistrons is not known

The degree of possible interaction between the 3'-terminus of 16S RNA from several different bacteria and the ribosome-binding sites of coliphage RNA is therefore consistent with the available data on translational specificity in these bacteria Confirmation of the hypothesis that the 3'-end of 16S RNA is directly involved in the selection of initiation regions on mRNA must await the sequencing of ribosome-binding sites from other bacterial messengers and direct demonstration of such an interaction For instance, we would predict that the sequences of the ribosome-binding sites from RNA of the *C crescentus* phage Cb5 would contain some part of the sequence complementary to the 3'-end of *C crescentus* 16S RNA, that is, some part of AGAAAGGA

Initiation factors

The role of initiation factors (for example, IF-3) in determining the efficiency of initiation on certain cistrons or mRNAs seems to be related to their capacity to stabilise the mRNA-30S subunit complex[1-3] 30S subunits, possibly through the 3'-end of 16S RNA and protein S12 (ref 12), have an inherent capacity to bind weakly but specifically to mRNA (ref 4) and are stabilised in this interaction by various initiation factors, particularly IF-3 (refs 1-3) and the ribosomal protein S₁ (ref 4)

The role of IF-3, which interacts with both 30S subunits and with mRNA (refs 1-3, 45), could be to potentiate or stabilise base pairing between the 3'-terminus of 16S RNA and the complementary region of the ribosome-binding site Purified IF-3 species promote differential translation of coliphage RNA cistrons[46-48], this may reflect a greater effect of IF-3 in the stabilisation of the mRNA-30S subunit complex at particular initiation sites Thus initiation of the coat-protein cistron of R17 RNA, where only three to four base pairs can be formed between mRNA and the 3'-end of *E coli* 16S RNA, may be highly dependent on the stabilising presence of IF-3 compared to initiation at the A-protein cistron, where seven such base pairs are possible Such a view is consistent with the effect of IF-3 on ribosome binding to unfolded MS2 RNA, where addition of IF-3 stimulates ribosome binding to the coat-protein site but not to the other sites[48] Similarly, crude initiation factors produce a significant stimulation in the binding of high-salt-washed ribosomes to the R17 initiator regions This effect is about elevenfold, eightfold and fourfold for the coat-protein, replicase and A-protein sites, respectively[26]

Table 2 3'-terminal sequences of 16S rRNA

E coli B	GAUCACCUCCUUA$_{OH}$ (ref 29)
P aeruginosa	G(X)₂PyCUCUCCUU(A)$_{OH}$*
B stearothermophilus	G(X)~₅PyUCCUUUCU(A)$_{OH}$*
B subtilis	G(X)~₇PyCUUUUCU$_{OH}$
C crescentus (ATCC 15252)	G(X)₃PyUCCUUUUCU$_{OH}$

Bacteria in mid-log phase were converted to protoplasts and extracted with a solution of phenol/cresol-aminosalicylate (see ref 29 and legend to Table 1) For all bacterial species examined, chromatography on DEAE-Sephadex of T₁-ribonuclease digests of terminally-labelled 16S RNA demonstrated the presence of a large ($\geqslant$12 nucleoside residues) 3'-terminal T₁-oligonucleotide X represents any nucleoside other than guanosine A more detailed account of the sequences and their determination is in preparation

*The variable presence of the 3'-terminal adenosine in 16S RNA is found in a variety of bacteria and depends on the culture conditions A more extensive examination of this phenomenon will be the subject of a separate report

Table 3   R17 ribosome-binding site sequences[6] and proposed pairing with the 3′-terminus of 16S RNA

Bacteria	R17 cistron	Possible pairing of 3′-terminus with appropriate region of ribosome-binding site sequence*		Number of base-pairs possible	Ribosome binding to unfolded R17, MS2 or f2 RNA (refs 2, 6, 8, 27, 28, 38)
E coli B		ₒₕ A U U C C U C C A C Py 16S RNA (ref 29)			
	A-protein	(5′) C U [A G G A G G U] U U(3′)		7	+
	Replicase	A C A U [G A G G] A U U		4	+
	Coat protein	A C C [G G G G G] U U U G		3(4)††	+
		↓ [A]†			
Ps aeruginosa		ₒₕ A U U C C U C U C Py 16S RNA			
	A-protein	(5′) C U [A G G A G] G U U(3′)		5	+
	Replicase	U G [A G G A] U U A C		4	+
	Coat protein	A C C [G G G G G] U U U		3(4)	+
		↓ [A]†			
B. stearothermophilus		ₒₕ A U C U U U C C U Py 16S RNA			
	A-protein	(5′) A U C C U [A G G A G](3′)		≥4	+
	Replicase	A C A U G [A G G A] U		4	+
	Coat protein	A A C C G G [G G] U U		2(1)	−
		↓ A †			
C crescentus		ₒₕ U C U U U C C U Py 16S RNA			
	A-protein	(5′) U C C U [A G G A G](3′)		≥4	See text
	Replicase	C A U G [A G G A] U		4	and ref 2
	Coat protein	A C C G G [G G] U U		2(1)	
		↓ A †			

*The sequence given represents that containing all or part of the conserved sequence 5′-AGGAGGU-3′ from the A-protein, replicase and coat-protein initiator regions of R17 RNA The initiator AUG is located eight to nine bases to the 3′-side of this sequence Apart from the G→A transition (see below), these sequences are identical to those available for the corresponding regions of f2 (ref 15) and MS2 RNA (refs 18, 21, 22 and 40)

†Two sequences have been reported for this region of the coat-protein binding site in the two different R17 stocks used[6,41], the G→A transition probably represents a spontaneous mutation which occurred after the two stocks were separated[41] Such a transition may have some selective advantage since it would increase the stability of interaction between the coat protein initiation site and the 3′-end of 16S RNA The corresponding sequence from MS2 (refs 22 and 40) and f2 RNA (ref 15) contains the A substitution The *in vitro* ribosome-binding data were obtained with R17 RNA containing the GGGG sequence[6,38] and with f2 or MS2 RNA presumably containing the GGAG sequence[2,8,27,28]

‡The apposition of a G and a U residue in the proposed helical region formed between the coat-protein binding site and 16S RNA would cause little, if any, destabilisation of the base-paired structure[42,43]

The bacterial protein, factor ι (interference factor), also modifies cistron selection in the translation of coliphage messengers by bacterial ribosomes Significantly, it seems to be identical to the fractional 30S ribosomal protein S₁ (ref 49), which can be linked chemically to the 3′-terminus of 16S RNA *in situ*[50] and which is necessary for the specific, initiation factor-independent binding of 30S subunits to mRNA (ref 4)

Factor ι is also identical to the α-subunit of the Qβ replicase complex[51], the presence of the α subunit is essential for the binding of the replicase to the 3′-terminus of the Qβ+strand[52] The 3′-sequence of this RNA is UCUCCUCCCAₒₕ (ref 53), and the 3′-sequence of 16S RNA is UCACCUCCUUAₒₕ, that is, they both share the sequence CCUCC which we propose to be involved in interaction with ribosome-binding sites As factor ι has a specific affinity for certain polypyrimidine tracts in RNA (ref 54), its complex regulatory function may relate to a capacity for recognising such nucleotide sequences in mRNA and at the 3′-terminus of 16S RNA

The inhibitory activity of factor ι on the translation of

coliphage RNA is restricted to initiation at the coat protein cistron[55] and is a function of mRNA concentration[54-,56] This effect may be the result of both a direct binding of factor ι to a region of the mRNA close to the coat protein initiation site[56], particularly at high concentrations of factor ι, or to a competition between the factor and mRNA for a ribosomal site[54], possibly the pyrimidine-rich 3′-end of 16S RNA It has recently been proposed that factor ι bound to the ribosome may be directly displaced by added mRNA (ref 54), the efficiency of which may depend on the sequence of nucleotides on the mRNA to which the ribosomes bind Thus initiation at the R17 coat-protein binding site, which can form only three to four base pairs with the 3′-terminus of 16S RNA, is inhibited by the presence of factor ι (refs 54–56), whereas initiation at the A-protein site (7 possible base pairs) is unaffected[55]

The ribosomal protein S₁₂ is also clearly involved in specific initiation on mRNA (ref 12) However, its role may result from its ability to interact with initiation factors on the 30S subunit[12], perhaps potentiating the specific recognition by the 3′-end of

16S RNA Using bifunctional cross-linking reagents it has recently been shown (Heimark, R , and Traut, R T , and Bollen, A , Kahan, L , Cozzone, A , Hershey, J W B and Traut, R R , quoted in ref 49) that S_1 (factor *i*), S_{12} and IF-3 give cross-linked products in the 30S subunit, and are presumably close to the 3'-end of 16S RNA, since S_1 can be chemically linked to the 3'-terminal nucleoside[50]

We therefore propose that the cistron specificity of bacterial ribosomes previously ascribed to the 30S subunit, can be further localised to a pyrimidine-rich stretch of about ten nucleotides at the 3'-terminus of the 16S ribosomal RNA We suggest that the degree of complementarity of this region with a purine-rich segment within the ribosome binding site sequence on bacterial mRNA determines the intrinsic capacity of the ribosome to translate a particular cistron The finer controls imposed on initiation at different cistrons may involve both the accessibility of the purine-rich segment to the 3'-terminus as determined by the secondary structure of the initiation region, and the availability of certain initiation factors which would modify the proposed interaction

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letters to nature

Submillimetre brightness spike at the solar limb

WE report here the first complete phase of the reduction of data obtained during the solar eclipse of June 30, 1973 from the high altitude moving platform provided by Concorde 001 Reports of the flight[1] and of an optical measurement of chromospheric thickness[2] are already published Submillimetre observations were made at both the second and third contacts, using a rapid-scanning Michelson interferometer At the second contact one complete interferogram of resolution 1 cm^{-1} was measured each second, but the limited passband of the detector, an InSb Rollin instrument[3], was found to have attenuated the highest frequencies (above 200 Hz) so these data have not been used At the third contact 10 s were taken per interferogram The instrument response was satisfactory, but the record needed subsequent digitisation, rendering analysis much lengthier, since an electronic analogue Fourier transformer had been built for 1 s interferograms, but was not usable for the 10 s data

Figure 1 shows a plot of solar flux against the lunar limb position in 3 passbands, centred at 400 μm, 800 μm, and 1,200 μm Radiation from the image of the revealed solar crescent, plus that from the lunar disk, and from the sky field

was plotted against time in each band Time was then correlated with the limb positions using aircraft coordinates and astronomical data The lunar and sky contributions were then subtracted, leaving the solar contribution as a function of the distance of the lunar limb from the solar optical limb Corrections for several effects were taken into account First, for atmospheric emission and absorption, the latter averaging less than 10% in the shortest, most obscured waveband Second, for spectrally selective absorption and emission by the 12 mm thick, crystal quartz, window of the aircraft Third, for spectrally selective absorption by the TPX lenses in the spectrometer and the TPX detector window Finally, for the wide lobes of the 12-cm telescope, which had 30' diffraction limited resolution at 1,200 μm The sky brightness temperature above 1,200 μm averaged 15 K above 57,000 feet, giving an accuracy limit of 1% of the total signal, which limited the relative radiometry

The separate contributions from the whole lunar disk and from the sky were measured with considerable precision during the approximately 65 min of submillimetre totality, by integrating alternately on and off the image of the lunar disk During the 7 min observing time starting at the origin of Fig 1 the power on to the detector from the Moon, within the instrument passband, was 3×10^{-8} W, from the sky it averaged $1\ 5 \times 10^{-8}$ W The power from the solar crescent when the optical
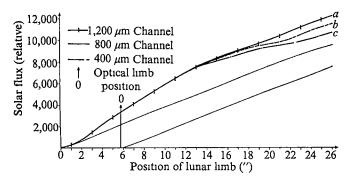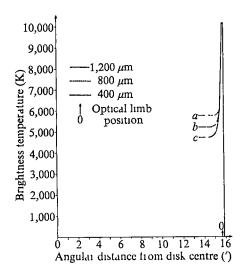
Fig. 1 Flux from the Sun against the lunar limb position in 3 submillimetre wavelength bands Error bars shown on the 1,200 μm curve apply closely to the other wavelengths Two lower plots show computed fluxes from theoretical models with no limb brightening Arrow indicates the position of the optical limb

limb was just revealed, at point 0, was estimated to be 1 5× 10⁻⁹ W The minimum detectable signal, expressed as the noise equivalent power resulting from the combined detector noise and the local emission noise was 8 × 10⁻¹² W Hz⁻¹ Thus, integration times of the order of seconds, and the dynamic range of 10³ to 1, yielded good signal-to-noise ratios in each of the 3 wavelength channels Nevertheless, a multichannel radiometer would have given superior values in this non-detector noise limited broad-band regime

Also shown in Fig 1 are computed curves for solar disks of a constant brightness temperature of 5,750 K, together with radii equal to the optical radius and 6" larger, respectively These show that there is an excess at the limb both geometrically and thermally distinguishable from 'flat-topped' distributions This is displayed clearly in Fig 2, which assumes circular symmetry about the centre of the solar disk The plots have a resolution, obtained by smoothing curves with an original 0 8" discrimination, of 1 6" That is superior by a factor of 25 to the resolution obtained, at 1,400 μm only, with the largest ground based millimetre-telescope[4], and by a factor of 50 to the resolution obtained in the two submillimetre wavebands[5] The brightness spike at the limb has been normalised to fit a brightness temperature, T_B at the centre of the disk, of 5,750 K in the 1,200 μm band A good fit to the

Fig 2 Brightness temperature against angular distance from the centre of the solar disk Dotted curves are partly extrapolated The solid curve, common to all three channels, is normalised at peak Arrow indicates the position of the optical limb

observations was then obtained by assuming the peak of the spike at 800 μm and 400 μm to have the same T_B value (10,100 K) as at 1,200 μm That leads to extrapolated disk temperatures at 800 μm and 400 μm of 5,200 K and 4,700 K, respectively, which are in reasonable agreement with previous measurements[6,7] As only 19" of photospheric disk were observable, however, these extrapolations rely heavily on a model which assumes that the Sun is a diffuse emitter except for the source responsible for the observed spike

The very limited angular extent of the excess emission, which extends from some 4" outside the optical limb, where it exhibits a very steep cutoff, to 5" within the optical limb, with a less steep decline, suggests that the spike can be identified with emission from chromospheric spicules This geometrical interpretation of the excess emission is corroborated by the apparently small change of peak temperature with wavelength Models of spicules from data at optical wavelengths[8], imply that each spicule is optically thick throughout the submillimetre range Though sparsely distributed, their superposition at the limb is here seen as the edge of a thin sheet at temperatures of the order of 10,000 K A more detailed analysis should yield information on the number density and length distribution of the spicules above the photosphere, in the region near the chromopause

Although an occultation experiment of this type is well suited to examining the large scale brightness distributions across the Sun, the limited time that was available after the third contact in this case has prevented such an examination here The mystery of the submillimetric limb brightening, missing on an arc minute scale[7,9] thus remains

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Circular polarisation in HDE226868

MUCH effort is being expended to develop an understanding of the details of X-ray production in some astronomical sources Accretion on to magnetic poles is the mechanism most often invoked for X-ray production in collapsed stars Strong magnetic fields, such as those associated with collapsed objects, are capable of producing observable circular polarisation, so it is of great interest to determine whether HDE226868, the binary containing Cyg X-1, exhibits this effect

Nikulin, Kuvshinov and Severny[1] reported circular polarisation from the light of HDE226868 that varied with time between 1 2% and 2 1% Subsequently, Gehrels[2], using a matching

Table 1 Time-averaged circular polarisation values for HDE226868

Filter	n	$(10^4) q \pm \sigma q$
U	2	$+ 1 7 \pm 4 1$
B	8	$+ 2 9 \pm 0 9$
V	1	$+ 4 0 \pm 3 0$
r	3	$- 5 1 \pm 0 9$
Infrared	1	$- 4 6 \pm 2 5$

colour response, observed no significant circular polarisation down to a level of about 0 05% Furthermore, he found no polarisation in the visible or ultraviolet regions down to levels of about 0 1%

Using the photoelastic polarimeter and 31-inch telescope at Battelle Observatory[3], we have obtained circular polarisation measurements of Cyg X-1 on nine nights during June–October, 1974 (Table 1) The fourth Stokes parameter V, normalised to unit intensity, is denoted by q, and n is the number of observations for a given filter Positive circular polarisation refers to counterclockwise rotation of the electric vector in a fixed perpendicular plane for an observer facing the source The effective wavelengths are 3,700, 4,200, 5,350, 6,450, and 7,250 Å for the U, B, V, r, and infrared filters, respectively The half-power transmittances are $\lambda\lambda$ 3,450–3,900 Å, $\lambda\lambda$ 3,800–4,750 Å, $\lambda\lambda$ 5,100–5,700 Å, $\lambda\lambda$ 6,000–7,200 Å, and $\lambda\lambda$ 6,750–7,800 Å
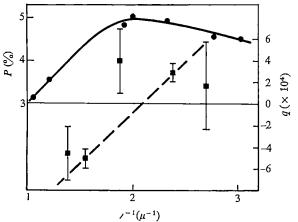
Fig. 1 Linear (●) and circular (■) polarisation of HDE226868 as a function of wavelength The linear polarisation is taken from Gehrels[2]

There is definite circular polarisation (≥ 3 σ) in two filters (B, r) The wavelength dependence of the linear polarisation, as presented by Gehrels[2] and plotted in Fig 1, suggests interstellar polarisation Coyne, Gehrels, and Serkowski[4] predict a maximum linear polarisation at about, 5,000 Å From calculations based on the analysis of Martin[5], we expect a circular polarisation near zero at this wavelength, and in the blue and red regions we expect approximately the same magnitude, but opposite handedness for q, this is roughly what is seen in Table 1 and Fig 1 Narrow-band measurements of both the linear and circular polarisation over the entire spectrum would help to separate interstellar and any possible intrinsic effects

The case for interstellar dust as the source of the circular polarisation is strong But the interest in this object warrants further polarimetric investigations

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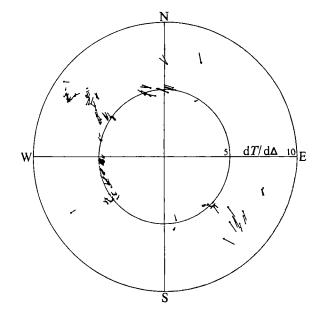
Evidence for mantle heterogeneity from two large seismic arrays

OBSERVATIONS from two large seismic arrays suggest that lateral heterogeneity is present throughout the Earth's mantle The differences between seismic event locations determined by the arrays and event locations determined by a global network of seismic stations indicate that seismic rays are deflected by velocity anomalies in the mantle

A large array can be used to locate seismic events because it provides a direct measurement of the azimuth and inclination (or wave slowness $dT/d\Delta$) of incoming teleseismic P-waves Such locations, however, are usually inaccurate because they are very sensitive to the effects of structure along the propagation path By contrast, locations determined by a global distribution of seismic stations and reference to standard travel time tables are accurate to within 30 km because the effects of small scale structure in the crust and mantle have been averaged out

Davies and Sheppard[1] suggest that large errors in event locations determined by the Large Aperture Seismic Array (LASA) must be attributed to lateral heterogeneity in the mantle If most of the heterogeneity is a long way from LASA, other arrays which sample the same source regions and approximately the same propagation paths should show similar mislocation tendencies I have determined locations for teleseismic events recorded at a large seismic network situated near Hanford, Washington The network contains 24 permanent stations, irregularly distributed within an area roughly 100×150 km All stations consist of 1-s vertical seismometers, modified to record higher frequency microearthquake activity This precludes detection of teleseismic events with magnitudes below 5 7, so that only about 200 acceptable events were recorded between 1970 and 1972 Two stations were eliminated because their travel time residuals show anomalously high scatter, the remaining 22 stations form the Hanford array Arrival times were chosen visually at the first coherent peak or

Fig 1 The Hanford array diagram The radial axis is $dT/d\Delta$ and the polar axis is array to event azimuth Each arrow represents a mislocation vector for one event, the head is the true location and the tail is the apparent location Mean vector 0 38 s per degree along azimuth 245 ° removed as described in ref 1

trough Reading accuracy is about 0 1 s which limits the accuracy with which events are located to ~ 200 km

The azimuth and $dT/d\Delta$ of incoming teleseismic signals were determined by a least squares fit of the best plane wave to the uncorrected data These quantities, together with the azimuth and $dT/d\Delta$ values predicted by the United States Coast and Geodetic Survey (USCGS) "preliminary epicentre" locations and reference to the Jeffreys–Bullen (JB) tables, were plotted on an array diagram (Fig 1) Seismic source regions can be located using Fig 2

A first order correction[1] for the effect of gross structural trends directly beneath the array has been applied to the data of Fig 1
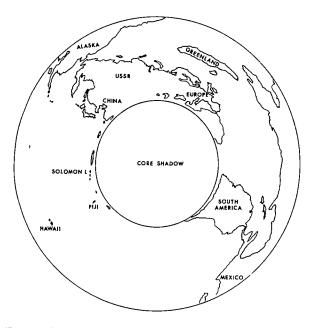
Fig 2 The coastlines of the world as viewed through an array diagram centred on the Hanford array, assuming that the JB tables for P-phases are correct This map was created by R Sheppard

The Hanford array mislocates most seismic events Systematic mislocations are associated with several source regions such as Russia, the Ryukyu, Bonin and Solomon Islands and the northern portion of South America Rapid variations in both azimuth and $dT/d\Delta$ anomalies are also present, notably associated with events from the southern end of the Solomon Islands, the coast of Chile and the island of Hokkaido

An array diagram for LASA is shown in Fig 3 The coastlines of the world as viewed through an array diagram centred at LASA are very similar to those shown in Fig 2 LASA also systematically mislocates events from several source regions, and the direction of mislocation is strikingly similar to that observed in the Hanford array diagram Some of these features are more easily seen in Fig 4

The similarities in the global pattern of mislocations for the arrays suggests that structure at considerable distance from the arrays is responsible for most of the mislocation The only alternative explanation requires nearly identical structure beneath both arrays which is unlikely My analysis supports the suggestion[1] that most of the mislocation is a result of lateral heterogeneity in the mantle

Azimuth and $dT/d\Delta$ anomalies associated with several source regions are too large to be attributed to structure associated with descending slabs of lithosphere[1–4] Most of the orientations of the mislocation vectors show no obvious correlation with the dip of the descending slab or with depth to source There is a tendency, however, for the orientations of the mislocation vectors to change from one trench region to the next, as shown by the data for the Hebrides and Solomon Islands
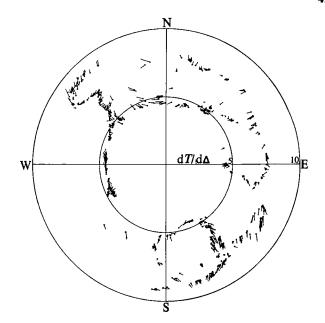
Fig. 3 An array diagram for LASA Data used to construct this diagram are the same as those used by Davies and Sheppard[1] Mean vector removed as in ref 1

(Fig 4) Similar effects are seen in the LASA data This suggests that structure close to the source regions must be responsible for at least part of the observed mislocation As the descending slab cannot be responsible, it seems that we are observing the effects of structure on deeper interfaces such as the 630 and 800 km discontinuities

Events from Russia seem to be arriving 5° off azimuth at both LASA and Hanford A significant difference should be observed between the average JB time residuals calculated for each array if the incoming wave fronts were deflected at great distance from the arrays, for instance in the deep mantle, and were not seriously distorted during their subsequent traverse to the Earth's surface The magnitude of the time
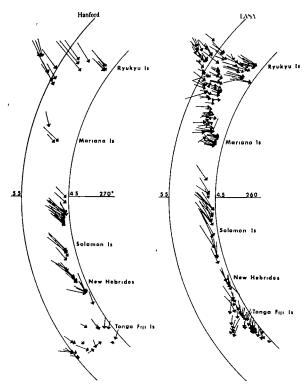
Fig 4 Enlarged portions of the LASA and Hanford array diagrams

difference is approximately $t = H\cos\theta\,\delta\theta/V$ where H is the distance between the arrays, θ is the angle between the line connecting the arrays and the wavefronts (if the wavefronts suffer no distortion) $\delta\theta$ is the azimuth anomaly and V is the apparent velocity of the waves as they sweep across the array For the Russian events, $H = 900$ km, $\theta = 6°$, $\delta\theta = 5°$ and $V = 22$ km s^{-1}, the expected time difference is about 3 5 s This has not been observed Even allowing for dissimilar $dT/d\Delta$ anomalies at each array and for variations associated with travel time data[5] it is extremely difficult to mask an expected time difference of 3 5 s This suggests that similar structure in the upper mantle directly beneath both arrays may be responsible for the mislocation of Russian events But the general agreement in the global pattern of mislocations at both arrays suggests that upper mantle structure beneath the arrays is not responsible for most of the features in the array diagrams

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Hydrothermal metallogenesis in the Bauer Deep of the south-eastern Pacific

SEDIMENTS from the East Pacific Rise that are unusually enriched with iron, manganese, and other metals[1,2,3] are correlated with areas of high heat flow on oceanic ridges With the discovery that sediments rich in iron directly overlie oceanic basaltic basement, it has been suggested that such deposits were formed by circulating hydrothermal fluids generated during mid-oceanic rift volcanism[4,5] Other suggested origins are authigenic precipitation of iron and manganese from seawater[6], submarine weathering of basaltic debris, and precipitation during diagenetic remobilisation of manganese
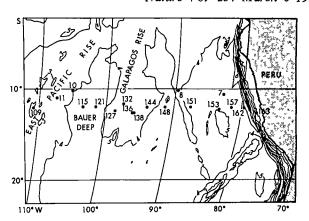
Fig. 1 Index and core location map showing general bathymetry in the 12°S region of the Nazca plate

in the sediment column[7] Our geochemical evidence supports the hydrothermal metallogenesis hypothesis

The Bauer Deep, located between the East Pacific Rise and a fossil spreading centre, the Galapagos Rise[8], is an area of sediment accumulation rich in metals (Fig 1) This sediment may originate from East Pacific Rise crest exhalations that are transported into the Bauer Deep[9] Heat flow values for the Bauer Deep are larger than values for other oceanic basins of the same age[10], the anomalously high heat flow values in the Bauer Deep with their large variability and bimodal distribution are characteristic of mid-oceanic ridge heat flow patterns Anderson and Halunen have suggested[10] that metal hydroxide precipitations formed by hydrothermal exhalations within the Bauer Deep produce the metal-rich sediments there This is borne out by our geochemical evidence based on element distributions and accumulation rates across the Nazca lithospheric plate

The surface layers (0 to < 10 cm) of 12 cores were analysed for metal content Chemical compositions were determined by X-ray fluorescence spectrometry and atomic absorption spectrophotometry On four of the cores, sedimentation rates were determined by the logarithmic decrease of unsupported ^{230}Th with depth in the sediment The core length over which the unsupported ^{230}Th activity decreased by one-half was divided by 75,200 yr, the half life of ^{230}Th This method has been successful on Quaternary pelagic sediments[11-13] Metal accumulation rates were calculated by multiplying the bulk sedimentation rate by the metal content, assuming an *in situ* dry uncompressed bulk density for pelagic sediments of 0 75 g cm^{-3} (ref 14)

Table 1 Location and composition of surface sediment

Core no	Latitude (S)	Longitude (W)	Depth (m)	CaCO$_3$ (%)*	Al$_2$O$_3$ (%)†	Fe$_2$O$_3$ (%)†	MnO (%)†	Cu (p p m)‡	Ni (p p m)‡	Co (p p m)‡	Cr (p p m)‡
109	12°05′	110°37′	3,069	90	0 97	0 13	0 14	97	89	35	37
11	11°00′	107°30′	3,507	90	0 96	0 12	0 14	120	73	30	33
115	11°58′	103°23′	4,548	7	5 72	19 44	6 33	750	1050	210	70
121	11°59′	101°31′	3,952	77	1 93	2 15	1 01	180	200	73	48
127	12°23′	98°45′	4,062	70	3 25	3 46	1 62	310	500	70	60
132	11°33′	97°27′	3,996	75	2 99	1 93	1 00	230	370	75	39
136	12°35′	95°48′	3,457	53	7 45	6 33	2 38	470	600	100	83
138	12°36′	95°42′	4,286	5	10 05	12 28	4 16	560	950	300	95
148	11°59′	91°19′	3,945	71	4 00	2 46	0 51	260	240	150	69
151	12°01′	87°33′	4,296	17	12 97	5 97	0 99	130	390	60	120
7	10°33′	83°02′	4,514	2	13 66	6 86	0 49	160	260	29	140
162	12°01′	79°34′	5,026	3	12 88	6 27	0 10	85	38	—	135

*Calculated from CaO determination by X-ray fluorescence spectrometry
†Al$_2$O$_3$, Fe$_2$O$_3$ and MnO by X-ray fluorescence spectrometry Coefficients of variation are less than 2%
‡Cu, Ni, Co, and Cr by atomic absorption spectrophotometry Coefficients of variation are less than 15%

Figure 1 shows the location of 18 cores analysed during a larger study of their chemical and mineralogical composition (G M McM , unpublished) The transition metal, aluminium and calcium carbonate contents of 12 of these cores are presented in Table 1 Figure 2 shows the distribution of iron, manganese and four trace metals on a profile across the Nazca plate at approximately 12°S The metal concentrations were normalised to aluminium in a method similar to that of Piper[15], who found that the metal concentrations of East Pacific Rise sediments showed more internal consistency and exhibited stronger maxima near the rise crest when treated in that way than when plotted on a carbonate-free basis

In the Bauer Deep, enrichment is greatest for iron, manganese, copper, and nickel (Fig 2) Iron and manganese distributions correlate well with each other across the entire area, but those of other metals do not Nickel and copper show greatest enrichment in the Bauer Deep and Galapagos Rise sediments, and some covariance with iron and manganese Cobalt and chromium show no enrichment and display no covariance with the other transition metals We interpret the strong iron and manganese covariance as evidence for coprecipitation of iron and manganese hydroxyoxides[9] The distributions of nickel and copper are similar to those of iron and manganese and those metals may therefore be fixed in the same manner But hydrothermally derived iron oxides may at least partially scavenge manganese and associated metals such as nickel and cobalt from seawater by catalytic oxidation[16] The distribution of chromium may be explained by its mobility in the oxidising environment of the sediments, as it forms soluble complexes in the hexavalent state[17] The cobalt distribution generally follows that of chromium, although greater enrichment would be expected because cobalt should not be as mobile as chromium in these sediments

The transition metal accumulation rates of four cores representing Quaternary sedimentation on the East Pacific Rise, the Bauer Deep, the Galapagos Rise, and on the plate near the
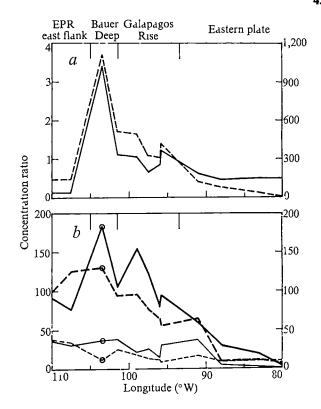
Fig 2 Distributions of Fe_2O_3, MnO, Cu, Ni, Co, and Cr in ratio to Al_2O_3 in surface sediments from the Nazca plate at approximately 12°S a ————, $MnO/Al_2O_3 \times 10^{-3}$ (right hand scale), —, Fe_2O_3/Al_2O_3 (left hand scale) b ————, Cu/Al_2O_3, —, Ni/Al_2O_3, ————, Cr/Al_2O_3, — Co/Al_2O_3, ○, Bauer Deep Sediment All figures in $b \times 10^{-4}$ EPR=East Pacific Rise

Table 2 Surface sediment accumulation rates

Core no	Region	Sedimentation rate (cm per thousand years)	CaCO₃—free sedimentation rate (cm per thousand years)	Accumulation rates (mg cm⁻² per thousand years)					
				Mn	Fe	Cu	Ni	Co	Cr
109	East Pacific Rise	1 08	0 11	0 88	0 73	0 08	0 07	0 03	0 03
115	Bauer Deep	0 19	0 18	6 98	19 4	0 11	0 15	0 03	0 01
132	Galapagos Rise	0 20	0 05	1 16	2 03	0 03	0 06	0 01	0 01
7	Eastern Plate	0 23	0 23	0 65	8 28	0 03	0 05	0 01	0 03

Peruvian coast are presented in Table 2 The bulk sedimentation rate across the plate is approximately 0 2 cm per thousand years except for the East Pacific Rise crest, which shows a higher rate attributable to carbonate deposition Bender et al[18] determined manganese accumulation rates for pelagic sediments and nodules They found that the manganese accumulation rates for pelagic sediments ranged from 0 2 to 3 2 mg cm⁻² per thousand years with an average value of 1 3 mg cm⁻² per thousand years But an accumulation rate of 35 mg cm⁻² per thousand years was found for a core taken farther south than ours on the East Pacific Rise crest[6] This rapid rate of accumulation may be further evidence that manganese and other transition metals are derived from ridge–crest volcanism Our data show that manganese is accumulating on the East Pacific and Galapagos Rises at 12°S at a rate close to the average pelagic value, but that in the Bauer Deep it is accumulating at more than five times that rate Accumulation rates for iron, copper and nickel are similarly higher in the Bauer Deep sediments than in those from the other areas

Our data indicate that metal-rich sediments can be derived locally in areas of great heat flow such as the Bauer Deep and need not be formed exclusively at an active spreading centre and transported elsewhere The transition metal to aluminium maxima found by Piper[15] for the East Pacific Rise crest and the

rapid manganese accumulation rate established by Bender et al[6] have been used as evidence for local volcanism as the source of these metals Our data provide a similar argument for the Bauer Deep iron and manganese would seem to be the direct products of volcanism A direct relationship with volcanism is not as clear for the other transition metals

In areas where the accumulation rates are close to those of average pelagic sediments, authigenic precipitation is probably a significant factor for metal enrichment, and submarine weathering must also make contributions in areas of slow sedimentation where authigenic minerals such as phillipsite are forming[7,19] Diagenetic remobilisation of manganese is unlikely, however, in oxidised pelagic sediments

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Fig 1 Variation of continental area within 30° of poles as a function of time The shaded parts of the time axis include all extensive glaciations[4,5]

Long term variations in the albedo and surface temperature of the Earth

THE surface temperature of the Earth depends primarily on the solar constant, the Earth's albedo and the total mass and chemical composition of the terrestrial atmosphere Studies of climate covering the past few million years have generally allowed for variations in albedo in calculating average values of the surface temperature But over longer periods of time, however, less allowance has been made for albedo variations, it has, indeed, frequently been assumed that the albedo, when averaged over a long enough time, can be taken to be constant (see ref 1) We wish to point out that, on the contrary, long term variations in the albedo can be expected to occur, and to produce significant changes in the average surface temperature

The total albedo of the Earth depends on the relative proportions and dispositions of the continents and oceans and their related cloud covers This varies both because the cross section presented to incident solar radiation by a given area depends on its latitudinal position, and because the variation in reflectivity with angle of incident radiation changes from land to ocean Throughout much of the geological record, the relative amounts of continent and ocean do not seem to have undergone major change, but the relative disposition of the two has varied considerably because of continental drift The amount of land area, as distinct from continental area, has certainly undergone fluctuations, but no systematic trend appears either in time, or relative to the disposition of the continents on the Earth's surface We have estimated the average surface temperature of the Earth using a computer program which allows both for the greenhouse effect of the atmosphere and for variations in albedo resulting from different positions of the continental masses, but which assumes no significant change in the ratio of land-to-ocean area (Surface temperatures have been computed as a function of latitude for land and ocean separately, and an average, weighted according to the areas involved, has then been calculated)

If cloud cover is initially ignored, the important factor is the latitudinal distribution of the continents We have therefore looked especially at two extreme cases (1) where the continents are gathered together into a belt round the equator, (2) where they form a cap round one, or both, poles We have constructed semi-empirical curves of reflectivity as a function of the angle of incident radiation, assuming in case (1) a covering of soil, and in case (2) an ice–snow covering Although considerable information is available on the albedo of different types of surface, data on the variation of albedo with solar elevation are less well determined[2]. Fortunately, computer runs using a range of possible albedo variations with angle indicate that only minor changes are produced in the surface temperature with any reasonable functional relationship The difference in albedo between the two models is found to change the surface temperature by more than 12 °C (the higher temperature corresponding to the equatorial position of the continents) Since the difference between a glacial and an interglacial period probably corres-

ponds to a change in average temperature of only 5 °C (ref 3), this albedo-dependent variation must be regarded as significant
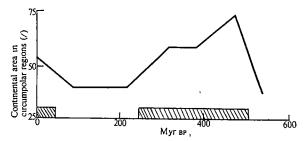
There are, however, two modifications that should be made First, we have assumed extreme dispositions of the land-masses in deriving this figure for the surface temperature More probable variations in the latitudinal position of the centre of area of the continents will still lead to differing values of the albedo, and therefore to differences in surface temperature, but the change will be smaller than that derived above Similarly, the effect of introducing cloud cover into the calculations, for any likely cloud distribution, is to reduce the change in albedo and so the variation in surface temperature Nevertheless, after allowing for both these factors, the amount of latitudinal change postulated in reconstructions of continental drift still seems from our estimates to be capable of producing a variation in surface temperature of a few degrees The effect of this would be to accentuate the prevailing climatic conditions In particular, although a land-mass near one of the poles would, in any case, be expected to have an ice–snow cover, a resultant slight lowering of the average temperature, as a result of the albedo effect, could turn this into a major glaciation The effects of this could then extend to appreciably lower latitudes than would otherwise be the case Whether or not this occurs will depend on other astronomical and geological factors What we can assert is a statement of probability that the likelihood of extensive glaciation occurring should be greater when a higher proportion of the land mass is near the poles

We have used the data cited by McElhinny[4] to derive values for the latitudinal distribution of the continents during each geological period The results are best represented in terms of the percentage of the terrestrial surface within 30° of each pole which is covered by continent The reason for choosing this form of presentation for the data is that continental displacements at lower latitudes, although leading to small changes in the albedo, cannot support extensive glaciation It is the latter that can most readily be detected in the geological record

Some of the data on continental drift remain uncertain In particular, information on continental drift and major glaciations in the Precambrian is still inadequate for the type of comparison we wish to make here Nevertheless, as can be seen from Fig 1, if we separate out the periods during which extensive glaciations most commonly occurred[5], these correlate reasonably well with periods when a higher percentage of the circumpolar regions was occupied by continents Within the limits of accuracy of the data, therefore, predictions based on the albedo effect are supported by the available geological evidence

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Recent changes in Atlantic surface temperatures

RODEWALD recently published brief notes about the temperature measurements of the sea surface made by nine North Atlantic weather ships during the past two decades[1,2] His remarks were prompted by the regrettable fact that several of these ocean stations are being discontinued, a decision which will make it much more difficult to monitor the temperature trends in the North Atlantic He presented[1] average annual temperature values for overlapping 5-yr periods, showing a distinctive decrease of 0 56 °C from the 1951–55 period to the 1968–72 period (Fig 1) Rodewald also provides[2] three important maps the changes in surface temperatures from 1951–55 to 1968–72
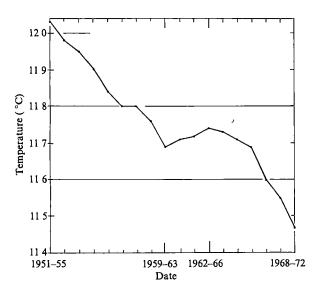
Fig 1 Running 5-yr mean surface temperature at the nine North Atlantic fixed station weatherships, according to Rodewald[1]

for the annual mean, and for the months of February and August Figure 2 shows his analysis of the February changes There is a remarkable consistency in the values, for example, at Station D (44°N, 41°W) the surface temperature declined by 1 61 °C in the annual average, by 2 12 °C in February and by 2 10 °C in August These are surprisingly large changes over such a short time span Since they are based on a well defined homogeneous record of careful observations there can be no doubt that they are real The core of decreasing temperature lies along the north edge of the Gulf Stream extension, suggesting a south eastward shift of that current and replacement with Labrador Current waters, especially in the vicinity of the Grand Banks The largest changes are encountered across the Atlantic between 40° and 50°N

Here we attempt to put Rodewald's maps into perspective by comparing them with maps of surface temperature change for other time intervals, so that one can assess the significance of the changes he depicts One of these may be obtained by subtracting the temperature pattern obtained by the CLIMAP group[3] for the most recent ice age (approximately 18,000 yr ago) from the recent pattern (Fig 3) The CLIMAP pattern was constructed by objective methods from the assemblages of fossil planktonic foraminifera in approximately 90 deep-sea cores

Values north of 50°N could not be determined with great accuracy as, according to the ice-age map, temperatures there are given as 'below +2 °C' The differences cannot, however, be much larger than approximately 12 °C near 45°N and less farther north, since the current temperatures at these latitudes are not higher than +12 to +13 °C The CLIMAP charts from which Fig 3 was derived clearly show that the long tongue of maximum anomaly along 45°N results from a southward shift of the maximum temperature gradient at the north edge of the Gulf Stream extension

In Fig 2, the values of the 22-yr change in February temperatures given by Rodewald are entered at the various weather ship station locations At Station D, for example, (indicated also on Fig 3) the 22-yr recent change of February surface temperature was −2 12 °C This station is located in the area of maximum change between the most recent ice age and today, the estimated change was about 11°C So it seems that we have experienced, in the past 29 yr or so, a change in Atlantic ocean temperatures which amount, in the Gulf Stream vicinity, to about one-sixth of the difference between total glaciation and our present climate
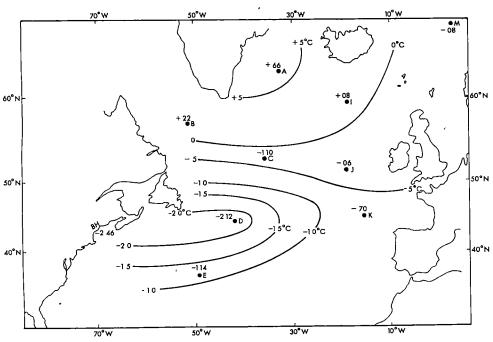
Fig 2 Change of February North Atlantic surface temperature from the 1951–55 pentade to the 1968–72 pentade, according to Rodewald[2] (Data for this figure would support an analysis even more consistent with that of Fig 3 than Rodewald's analysis)
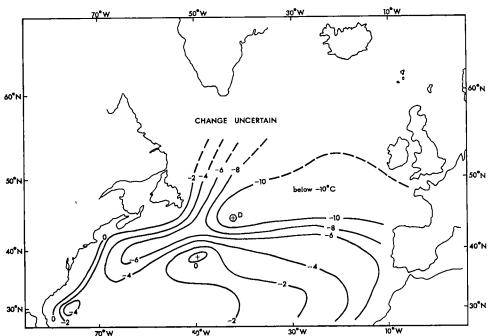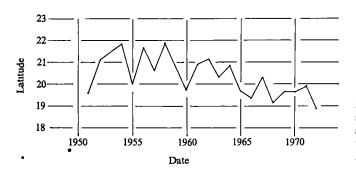
Fig. 3 Difference between ice age surface temperatures, (approximately 18,000 yr ago) and present winter surface temperatures, derived from the CLIMAP reconstruction[3]

Another comparison may be made with the map of average North Atlantic surface water temperature change between the early 19th century and the 1887–1899, 1921–38 period given by Lamb[4] Rodewald's figures approximately mirror those given by Lamb, indicating that the change from 'Little Ice Age' conditions to the early part of the present century was just about wiped out between 1951 and 1972 The 'Little Ice Age' thus seems to have been a return of about one-sixth of the way to a full ice age Rodewald's map in turn indicates that, at least as far as surface temperatures in the North Atlantic are concerned, the pattern had returned roughly to 'Little Ice Age' conditions from its early 20th century excursion by 1972

Two other pieces of information tend to confirm the relative magnitude of the surface temperature change given by Rodewald First, in West Africa during the same 1951–72 period, the northern edge of the monsoon drifted irregularly southward, as indicated in Fig 4 Assuming that if this displacement persisted at the 1968–1972 latitude the biotic zonation would adjust similarly, a comparison may be made with the late glacial zonation as given by Cloudsley-Thompson[6] Once again the 1951–72 shift seems to have been about one-sixth of the late glacial to present displacement

The second observation is the change of worldwide annual average snow and ice cover reported by Kukla and Kukla[6] In the northern winter of 1971–72 there was a rapid increase of snow and ice cover which persisted into the following years This increase was also about one-sixth of the difference between present-day conditions and those at the end of the most recent glaciation

We do not have an adequate basis for estimating whether or not the changes observed in the 1951–72 period will continue to grow, but it is essential to monitor further development with great care Indeed, we do not know whether the pattern of 1974 represents an end to the trend of the previous two decades or simply a temporary reversal like those which have characterised individual years of those decades on occasion But we should not assume that the change indicated by Rodewald's data is just a short term passing anomaly easily reversed in a year or so

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Fig. 4 Northward penetration of the monsoon rains in West Africa in the longitudes of Nigeria The higher the latitude to which the rains penetrate, the greater the seasonal rainfall The latitude of the northern edge of the monsoon rains was obtained by least squares fitting of a trend line to the rainfall amounts at various stations in the longitudes of Nigeria The intercept of this line with zero rainfall was taken as the northern edge of the monsoon Few stations north of this calculated position reported more than a few millimetres of rainfall Plotted points are averages of the July and August values

Buckle propagation in submarine pipelines

DURING its construction a submarine pipeline is at the same time bent and loaded by the external pressure of the sea If it is bent too far, it buckles If the local external pressure is small, the buckle is limited to a short kink on the compressed side of the pipe But if the external pressure is large enough, the local bending buckle can transform itself into a buckle of a different form, which once initiated can propagate along

the pipe, driven by external pressure alone, even into regions free of bending. Figure 1 shows a pipe along which a buckle has propagated in this way. The mode can be observed by grasping a tube of toothpaste firmly between thumb and forefinger, and then moving the fingers along the tube. In a short transition region, between the fingers, the pipe bends from a circular into a dumb-bell cross section.

This problem is important because of the possibility that a mishap during pipelaying might initiate a buckle which would run along the completed section of a pipeline, perhaps for many kilometres, at a cost of the order of $1.5 million a kilometre. Little has been published about this problem[1]. We describe the results of a preliminary analysis and of some tests on model pipes, and concentrate on determination of the minimum pressure needed just to sustain propagation. Buckle

Fig. 2 Dimensionless propagation pressure $p(R/t)^2/Y$ as a function of R/t. Points indicate experiments on stainless steel tubes of diameter 50.8 mm (■) and 12.7 mm (▲). — — — —, Predicted by simple theory.
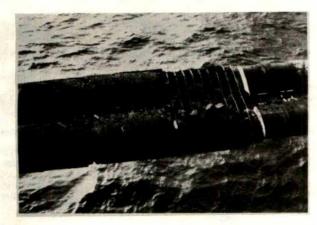
Fig. 1 Pipeline collapsed by a propagating buckle. The test was carried out on steel pipe of outside diameter 813 mm and wall thickness 19.1 mm.

propagation of this kind is distinct from the classical phenomenon of elastic buckling of cylinders under external pressure[2]; in a typical pipeline the propagation pressure is about a third of the elastic critical pressure.

A pipeline is essentially a steel tube with radius:thickness ratio of the order of 20, cold formed from a medium strength ductile steel and surrounded by a concrete weight coating (which contributes little to its strength). It can be treated as a thin shell. We begin by considering the kinds of deformation such a shell can undergo. It can either deform inextensionally, in a mode which bends the middle surface but does not stretch it, or extensionally, in a mode which both stretches and bends the middle surface. In general, the amount of energy required to deform the shell in an inextensional mode is much less than that to produce a deformation of similar magnitude in an extensional mode[3]; the shell 'prefers' to deform inextensionally if it can. Rayleigh showed, however, that if a cylindrical shell is constrained so that one circumference undergoes no deformation, then no purely inextensional deformation is possible[3]. Although there is thus no way in which the buckle can propagate without any stretching of the middle surface, it will develop a buckle configuration in such a way that the pipe is distorted by bending as much as possible and stretching as little as necessary.

An estimate of the propagation pressure can be made by assuming a buckle to move along the pipe in an unvarying mode (so that an observer moving with it would always see the same buckle configuration), and equating the work done by the external pressure on the consequent volume change to the work required to deform the pipe. Since the deformation is so large, plastic deformation dominates over the effects of
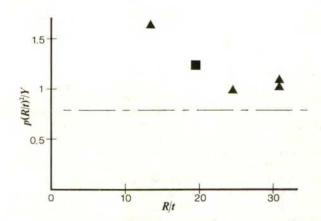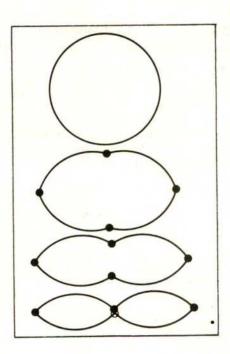
elasticity, so that the pipe deforms almost as it would if it were made from a rigid-plastic material. Then if the mode of deformation is purely inextensional, the propagation pressure p will be proportional to Yt^2/R^2, where Y is the yield stress, t the wall thickness of the pipe and R its radius. Different pipes made from different materials can then be compared by evaluating the non-dimensional propagation pressure $p(R/t)^2/Y$, which will be a constant if extensional effects are not important, and a function of R/t if they are.

Figure 2 shows the results of tests in which a buckle was driven along steel tubes. It shows that different pipes may be compared in this way, and suggests that the presence of extensional deformation exerts little influence for R/t greater than 20.

A theoretical estimate of the propagation pressure can be made from the work calculation described earlier. Suppose a cross section of the pipe to deform in the way shown in Fig. 3. All the deformation is in bending, concentrated into four 'plastic hinges'[4], separated by rigid regions. Two opposite hinges move together until they meet, and the other two move

Fig. 3 Mode of collapse assumed in analysis.

apart. If the material is perfectly plastic, the work calculation gives

$$p(R/t)^2/Y = \pi/4$$

Comparison with Fig. 2 shows that this somewhat under-estimates the observed pressure. Work calculations of this kind usually overestimate plastic collapse loads, and indeed can be proved to do so in the case of small deformations. Here the deformation is not small, and the buckle must also obey an internal equilibrium condition. If the transition from the circular to the dumb-bell cross section were very long, extensional deformation would be negligible and the total work dissipated would approach that corresponding to the above equation. But more detailed examination of the forces within the pipe wall shows that a buckle with such a long transition cannot propagate, because its equilibrium would require axial forces too large for the pipe wall to carry. Instead the transition shortens until the equilibrium conditions can be met: this involves a steeper transition, more stretching deformation, and a larger propagation pressure.

Other modes than the one shown in Fig. 3 can be used in the analysis, but this one gives the lowest propagation pressure. Its final cross section agrees quite well with the one observed in experiments.

One way of avoiding the danger of pipeline collapse by buckle propagation is to design the pipe so that its propagation pressure is greater than the greatest external pressure the sea can exert. It may instead be more economical to use a thinner wall but to guard against the possible destruction of long sections of line by placing at regular intervals 'buckle arresters' which will stop buckles which have begun to propagate. The design of buckle arresters will be discussed elsewhere.

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Xenopus from the Palaeocene of Brazil and its zoogeographic importance

THE African frog *Xenopus* (a member of the family Pipidae), formerly found in Miocene[1,2] and Recent deposits in Africa, is described here from the Palaeocene ($\sim$ 60 Myr) of Brazil. Extant pipids are aquatic freshwater frogs, occurring in sub-saharan Africa (*Xenopus, Hymenochirus, Pseudhymenochirus*) and northern South America (*Pipa, Protopipa, Hemipa*). They display a mosaic of primitive and specialised characteristics and are generally placed as an ancient offshoot of early anuran stock. Both fossil and recent occurrences of the Pipidae are consistent with an ancient distribution exclusively across the southern continent[3,5].

The Palaeocene material from Brazil consists of well pre-served, uncrushed braincase and postcranial elements, including those diagnostic of *Xenopus*—relatively narrow, flat, and un-sculptured skull roof bounded by parasagittal crests; well defined parietal foramen; paired or unpaired vomers, occa-sionally toothed; three or occasionally four acoustic foramina in the medial braincase wall; dagger shaped parasphenoid, lateral wings absent; only the inferior perilymphatic foramen present; cleft scapula; fused scapula and clavicle; iliac shaft lacking crests.

The morphological characteristics (expanded ear capsules, azygous frontoparietal, presence of parietal foramen, bilobed trochlear articulation of humeral head, far lateral position of the inferior perilymphatic foramina, short scapula, opistho-coelous (probably epichordal) vertebrae, fused sacrum and urostyle, and sacrum with widely expanded diapophyses) indicate that this frog is a member of the family Pipidae. The general head shape and the listed diagnostic features confirm our reference to the distinctive genus *Xenopus*, limited today to subsaharan Africa. Extant South American pipids and the African *Hymenochirus* are characterised by broad flattened skulls with a generally extensive supraorbital shelf; anteriorly wide parasphenoid; the presence of only the superior perilymphatic foramen and only a single acoustic foramen; a separate clavicle and scapula; and widely expanded iliac shafts. The distinctive morphology of pipids in general and of *Xenopus* in particular leaves no doubt about the identification.

Classification and description of Brazilian specimens
Class Amphibia
Subclass Lissamphibia
Order Anura
Superfamily Pipoidea
Family Pipidae
Xenopus romeri, n. sp.

Holotype: DGM (Divisão de Geologia e Mineralogia) 568, brain-case and sphenethmoid region of skull.

Paratypes: DGM 569, partial skull; 570, braincase; 579, ethmoid region; 575a-e, vertebrae; 576, fused first and second vertebrae; 573, sacrum with fused urostyle; 571, scapula-clavicle; 572, humeri; 577-578, ilia.

Locality: fissure deposits in limestone in the quarry of the Com-panhia National de Cimento Portland (Mauà), São José de Itaboraí, Estado Rio de Janeiro, Brazil[4]. Specimens collected in 1968.

Age: late Palaeocene.

Etymology: the species name honours the late Professor Alfred Sherwood Romer for his work on relationships of African and South American fossil faunas.

Diagnosis: species of *Xenopus* with robust, widened, well ossified skull with extensive fusion among braincase elements; cultriform pro-cess of parasphenoid fused or unfused; nasals co-ossified, may or may not be fused to frontals; parietal foramen opening far anteriorly on frontal; retractor bulbi muscle scars not excavating the paras-phenoid ventromedially; a well defined foramen for internal carotid artery present anterior to palatine foramen; a well ossified prepalatine connection separating palatine foramen from pro-otic foramen; three acoustic foramina (four in one specimen); condyloid fossa relatively well developed, with inferior perilymphatic foramen opening posterio-ventrally into the latter and separated dorsally from jugular foramen by a distinct roof or crest; parasagittal crests relatively far apart and meeting posteriorly almost at posterior border of frontoparietal; dorsal protuberance of ilium relatively small; first and second vertebrae fused; zygapophyseal surfaces simple.

Description: description here includes only those features necessary to confirm identification, or those that are of special anatomical interest. In DGM 568 (type) nasals and vomers are lost, not having been co-ossified (Fig. 1); in DGM 569 vomers and nasals are fused to the skull (Fig. 2), with faint or no indication of sutures.

The trigeminal foramen is separated ventrally from palatine fora-men by well ossified prepalatine connection; anterior to the palatine foramen a well ossified pillar of bone sets off an internal carotid foramen ventrally. On the medial braincase wall (visible laterally on 569 because of breakage) three prominent acoustic foramina occur (four on 569, see Fig. 2C); dorsal to the acoustic foramina a promi-nent endolymphatic foramen occurs. In occipital view inferior perilym-phatic foramina open posteriorly, separated dorsally from jugular foramina by distinct crests.

Vertebrae opisthocoelous; centra flattened, probably epichordal. Identified specimens include vertebrae 1-2 (fused), 3-6, 8-9; urostyle fused to sacrum; sacral diapophyses strongly expanded. Scapula and clavicle fused; scapula short, a prominent cleft present. Humerus with well ossified proximal end showing trochlear articulation characteris-tic of pipids, distal ends broken. Ilia with long, subcylindrical shafts, low rounded dorsal protuberance, prominently crested acetabulum,, and strong interiliac symphysis region.

Interrelationships among extant species of *Xenopus* have not been studied extensively; it is a relatively well defined, compact genus but there are few known osteological features that can be used to separate each species. Lengthy comparisons cannot be made here, but the Brazilian fossil *Xenopus* shows no special resemblance to the extant species *X. laevis, X. clivii, X. mulleri, X. fraseri,* and *X. gilli* (material of *X. kisigiensis*[5] was not available for this study).
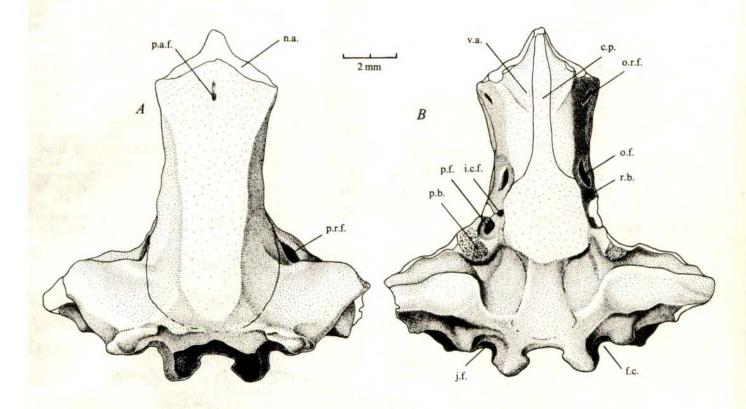
Fig. 1 *Xenopus romeri*, n. sp., skull of holotype, DGM 568. *A*, Dorsal; *B*, ventral; *C*, occipital. a.f. acoustic foramina; c.p., cultriform process of parasphenoid; d.p., dorsal prominence of ilium; e.f., endolymphatic foramen; f.c., condyloid fossa, f.c.l., fused clavicle; f.v., fused vomer; i.c.f., internal carotid foramen; i.p.f., inferior perilymphatic foramen; j.f., jugular foramen; n.a., nasal articulation surface; n.f., fused nasals; o.f., optic foramen; o.r.f., orbitonasal foramen; p.b., pseudobasal articulation; p.a.f., parietal foramen; p.c., prepalatine connection; p.f., palatine foramen; p.r.f., pro-otic foramen; r.b., retractor bulbi muscle scars; t.f., trochlear foramen; v.a., vomerine articulation surface.

So far as fossil *Xenopus* are concerned, *X. romeri* and the Miocene *X. stromeri* from South-west Africa[1] both have the parietal foramen in a far anterior position and relatively small occipital condyles, so far as may be judged from the rather poor figures of the latter (unfortunately the materials of *X. stromeri*, deposited in the Humboldt Museum in Berlin, have been lost). More significantly, *X. romeri* resembles an unnamed Miocene *X. tropicalis*-like form from Morocco[2] in having a relatively short centrum and closely-spaced glenoid cavities of the fused first and second vertebrae. These conditions are of special interest as there are a number of resemblances between *X. romeri* and the living *X. tropicalis* from western Africa; a greater number than with other living or fossil species. In general, the length–width proportions of the braincase region (especially the greater width of the braincase at the level of the orbitonasal foramen), the degree of fusion and the extent of ossification of the braincase elements (including the dermal elements), the posterior extent of skull table, the position of retractor bulbi scars, and the fusion of the first two vertebrae, indicate a similarity or close resemblance between the two species.

Although *Xenopus romeri* has a number of unique features that set it aside from other species of *Xenopus*, the number of resemblances to *Xenopus tropicalis* suggests that there is an actual relationship between *X. tropicalis* and *X. romeri*. Vergnaud-Grazzini[2] has suggested that the similarities to *X. tropicalis* shown by her Miocene Moroccan specimens are convergent rather than indicative of relationship. Since *X. romeri* seems to bridge the gap between the Moroccan fossils and the extant *X. tropicalis* in the configuration of the fused first and second vertebrae, it is possible that these three groups

may be more closely related *inter se* than to other species of *Xenopus*.

The presence of the now extinct *Xenopus romeri* in South America is of considerable zoogeographic interest. It is compatible with the present plate tectonic theories: South America and Africa did not separate completely until sometime in the Cretaceous[6,7]. The presence of *X. romeri* in South America and its close resemblance to the extant western African species *X. tropicalis* is strong biological evidence for a past connection between the two continents. Similar (although weaker) evidence from the same locality in Brazil is offered by *Apodops pricei*, the first known fossil caecilian amphibian, the relationships of which seem to be with a living genus from western Africa[8].

Knowledge of the southern (Gondwanan) radiation of pipids thus forms a more coherent picture. *Xenopus* is known now from South America as well as from Africa, and both of these continents also include forms that show intermediacy between *Xenopus* and the extant South American pipids: the Cretaceous *Saltenia*[9] in South America and the living *Hymenochirus* in Africa[10]. These resemblances cannot be explained by the migration of several now extinct and unrepresented phyletic lines through northern continents; they are clearly remnants of distribution on a single continental mass.

Both of the Miocene occurrences of *Xenopus* in Africa indicate that groups resembling the extant *X. mulleri* (*X. stromeri*) and *X. tropicalis* (the Moroccan fossils) were well established by that time. The resemblance of the latter two species to the Palaeocene *X. romeri* indicates that radiation within *Xenopus* must have preceded separation of the African and South American continental plates. There exists some minor disagree-
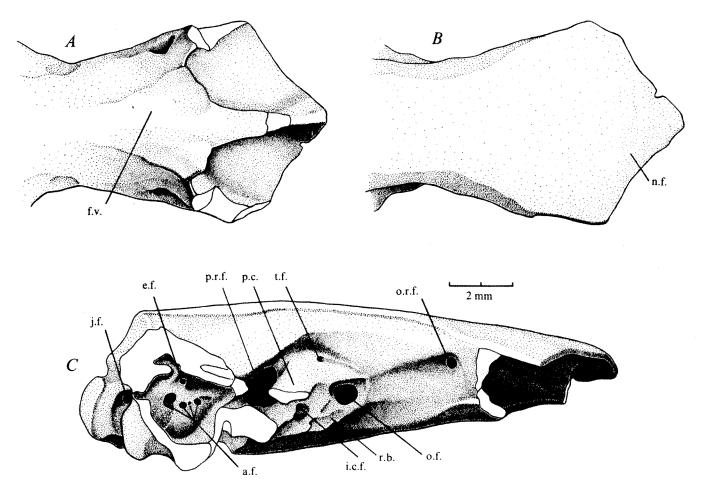
Fig. 2 *Xenopus romeri*, n. sp., DGM 569. *A*, ventral view of snout; *B*, dorsal view of snout; *C*, right lateral view of skull. Abbreviations as in Fig. 1

ment with regard to the timing of the separation, but available evidence indicates that a date of 120 Myr (early Cretaceous: Neocomian) for the onset of plate separation is consistent with a date of 90 Myr (late Cretaceous: Turonian) for the actual separation of the continents by a water gap. By about 70 Myr ago (late Cretaceous: Maastrichtian) there seems to have been a gap large enough to have prevented east–west migration of marine reef colonies[4]. *Xenopus* tadpoles can tolerate some salinity[11], but in view of the aquatic habits of the genus such tolerance would probably not be sufficient to permit migration over an oceanic barrier, even on a large raft of floating vegetation. It thus seems possible that species of *Xenopus* not much more primitive than *X. romeri* (and therefore closely related to extant species) had developed by at least 70 Myr ago, and possibly as far back as 95 Myr ago[12].

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Physical basis and ecological significance of iridescence in blue plants

MANY terrestrial plants of lowland tropical rainforests exhibit a conspicuous blue-green iridescence on their leaves—Richards[1] has observed these plants in Africa, South America, and South-east Asia. We have seen many species of blue plants on the rainforest floor in Malaya, from diverse groups including the ferns, *Selaginella*, and flowering plants. In Malaya the most spectacular and most common iridescent blue plant is *Selaginella willdenovii* (Desv.) Spring, also frequently cultivated in greenhouses. All comments on the iridescent colour refer back to the observation of Stahl[2] who reported the presence of reflection granules in the epidermal cells of *S. willdenovii*.

Although there has been ample research on iridescent colour in animals, virtually nothing has been done with plants. The iridescent green colour of the cave moss, *Schistostega*, has been explained by the presence of peculiar epidermal cells which act as refractive lenses, focusing light on specially

oriented chloroplasts[3] Here, we propose an explanation for the iridescent blue colour in plants (*S willdenovii* in particular), provide evidence supporting our hypothesis, and speculate on the physiological and ecological significance of this iridescent blue colour in shade-tolerant herbaceous tropical plants

Stahl's early conclusions about plant iridescence can be refuted by simple observation First, the existence of granules reflecting blue light cannot be verified by microscopic observation Secondly, the iridescent colour disappears when the leaves are immersed in water Therefore, the colour must be an optical effect of the leaf surface and not of the internal structure The two optical phenomena that can provide a physical basis for this effect are diffraction and thin-film interference, both of which have been invoked to explain the iridescent colouring of many animal cuticles[4,5] Diffraction effects can be ruled out in the present case because there is no dispersion, reflected blue colour is constant for white incident light over a wide range of angle of incidence Furthermore, microscopic examination has revealed no surface features that could function as a grating Therefore, we suggest that the iridescent blue colour in *S willdenovii* is caused by a thin-film quarter wavelength interference filter on the upper cell wall of the epidermis This would account for previous observations, and direct experimental support has been obtained by analysing the reflectance of light from the leaves The spectral analysis was carried out using a Beckman Acta V spectrophotometer with total fluorescence attachment An opaque blank was used to project a monochromatic beam of light on to the leaf (at an incident angle of 60°) and to measure its intensity reflected (at the same incident angle) into the photocell A slit programme and a wavelength interval of 300–700 nm were used for all measurements
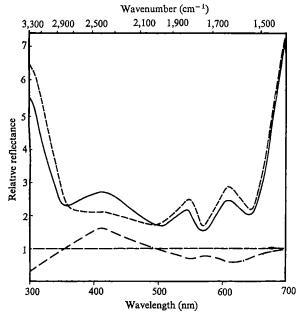
Fig. 1 Reflectance spectra of *S willdenovii* leaves Reflectance of the iridescent leaves is indicated by the solid line, of the non-iridescent leaves by the dashed line, and the difference between the two by the dashed and dotted line The horizontal dashed line indicates wavelengths of enhanced reflection or transmission Reflectance units are relative, and based on response of the spectrophotometer on a scale of percentage transmission

When iridescent leaves age or are exposed to sunlight for some time they lose their iridescence and develop an ordinary green appearance Chlorophyll content was found to be the same in both types of leaves (12 mg per g dry weight, by a colorimetric determination) We measured the reflectance of both iridescent and non-iridescent leaves, and plotted a difference spectrum (Fig 1) The difference spectrum shows maximum enhanced reflectance at 405 nm, a null point at

500 nm, and decreased reflectance at longer wavelengths, the effect was obscured above 660 nm by the strong reflective characteristics of all leaves[6,7] This curve corresponds closely with the operation of a quarter wavelength interference filter Precise analysis of the curve is only possible if the refractive index (*n*) of the filter is known We were not able to determine this, but estimate the filter thickness at 135–150 nm (assuming $n = 1\ 4\text{–}1\ 5$) Electron microscopic analysis would verify the existence of such a structural layer on the wall surface Solubility experiments suggested that cuticular waxes were not acting as the filter, the structural basis may well lie in cellulose orientation at the wall surface

General field observations suggest the functional significance of iridescence in certain plants Iridescent blue plants from distantly related groups grow exclusively in extremely shady tropical forest environments Furthermore, we have observed that leaves of *S willdenovii* lose their iridescence when they grow in sunlight, even when shade-green leaves of the same plant have the iridescent colour The light climate on the floor of the rainforest is extremely limiting for plants, the light intensity is low and deficient in wavelengths important for photosynthesis[10-12] The ecological importance of an interference filter in these circumstances is that the increased reflection of photosynthetically less active light (400–500 nm) is accompanied by increased penetration in the most photosynthetically active range (600–680 nm), this would have definite adaptive value Finally, preliminary observations on the leaf anatomy of *S willdenovii* indicate that the epidermal cells of these plants—egg-shaped with convex outer surface and chloroplasts in a peculiar position distal to the surface—may also function as lenses in a manner similar to that found in the moss *Schistostega*[3] Thus the analogy of a camera with coated lens may aid our understanding of the function of the leaf surfaces in these iridescent plants

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Sex recognition pheromone in tsetse fly *Glossina morsitans*

ATTEMPTS to identify a sex attractant substance in tsetse flies have failed because there seems to be no olfactory agent for sex recognition[1,2] Males are apparently sexually activated only by movement of the female, but the adverse effects of naturally occurring low population densities[3] on mating frequency is overcome as both sexes encounter one another at relatively high density around host animals[1] Experience in the laboratory confirms that sexual arousal in male tsetse flies, *Glossina morsitans morsitans* (Westwood), is initiated by movement of other individuals Thus, mature adult males will initiate sexual behaviour in the absence of females and the level of activity is increased if the flies are disturbed This behaviour ceases, however, as soon as physical contact is made with the target

male It seems unlikely that a sexually aggressive male would rely on rejection or acceptance by its target in order to distinguish females of its own species, and in view of the discovery of a short range sex pheromone in the housefly *Musca domestica* L [4-7], an attempt has been made to demonstrate the existence of a positive sex recognition pheromone in *G morsitans*

Observations made during the routine mating of one male to one female in a glass tube show that pairing in *G morsitans* is achieved very rapidly The problem of eliminating possible sources of sex recognition related to female behaviour (characteristic movements, sound production, or scent production occasioned by the presence of the male) was solved by testing the responses of sexually mature males (> 7 d old), individually in tubes, to decoys consisting of dead flies of various ages and nutritional states, recovered from our colony at Langford Male responses were measured subjectively an attempted copulation was scored if the male mounted the decoy, flexed its genitalia, and attempted to orientate to the copulating position a successful copulation was scored if the aggressor engaged its genitalia with the genitalia of the decoy This classification of responses was necessary as in later experiments dead males and inanimate decoys were used, and successful copulations were impossible A passing interest such as that observed when a male attacks another live male was not counted as a mating attempt Both the male fly and the decoy were replaced for each successive test Table 1 shows clearly that dead females were attractive to sexually mature males, and that their attractiveness was not diminished by low temperature storage or vacuum drying The attractiveness of dead females, however, was progressively reduced by washing in a variety of solvents, and was abolished by non-polar solvents

On the supposition that a chemical substance in the female cuticle provided the cue for continued male sexual activity on contact, and that this substance was removed by non-polar solvents, attempts were made to recover the substance from solvent washes and to test its activity with a suitable bioassay Mature male flies were again tested against decoys consisting either of dead males or of 'pseudo-flies' made from black shoelace knots[8], both of which were demonstrably unattractive to the mature live males Female and male *G morsitans* (immobilised by chilling), or females and 'pseudo-flies' were added to diethyl ether or hexane (0 5 ml per fly) in the ratio 4 1 and shaken The solvent was then evaporated *in vacuo* at room temperature The decoys were allowed to dry completely for several hours at room temperature in still air, whereupon they became highly attractive to mature male *G morsitans*, and maintained their attractiveness for at least 4 d This implies that a sex recognition pheromone of lower vapour pressure than the solvents used had been transferred from the female flies to the decoys

A specific sex pheromone for male houseflies has been isolated from the cuticular hydrocarbon fraction of adult flies, and olfactometer tests have shown it to be attractive over short distances[4-7] In view of the fact that the tsetse pheromone reported here was contained in the non-polar cuticular lipid fraction of female flies and that it seemed to be attractive to

males only over very short distances, we compared the distribution of hydrocarbons in the cuticle of female *G morsitans* with that of houseflies

Using the methods of Carlson *et al*[6], lipid samples obtained from cuticular rinses with hexane, of adult female *G morsitans* were fractionated using liquid chromatography on silica gel The hydrocarbon fraction was subjected to temperature-programmed gas chromatography on a 3% methyl silicone column (1 8 m × 2 mm) Table 2 shows a considerable difference in cuticular hydrocarbon composition between *G morsitans* and housefly

The distribution of hydrocarbons having 30 carbons or less was 93,90 and 79% for three housefly samples and only 6 8% for *G morsitans*, with C31 and C38 being most prevalent in the latter This striking difference gives rise to speculation about differences in cuticular composition in tropical insects such as tsetse flies since the C38 hydrocarbons of *G morsitans* are less volatile than the predominant C27 compounds in houseflies

The principle of using relatively non-volatile solvents as 'keepers' to retain volatile solutes is well known and has been practised by Beroza *et al*[9] in field trials with sex pheromones of several species of Lepidoptera Thus a sex attractant material dissolved in a less volatile solvent could be 'kept' better than one dissolved in a more volatile solvent On this basis, our experimental evidence for the existence of a sex recognition pheromone in *G morsitans* which is essentially a contact stimulant is supported by the gas chromatographic analysis of cuticular hydrocarbons of this species Alternatively, the *G morsitans* pheromone may itself be a compound with a high carbon number and a low intrinsic volatility This seems likely in view of its persistence in maintaining decoys in an attractive state for several days

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Table 2 Gas chromatographic analysis of hydrocarbons of female flies

Carbon no	M domestica*[*]	M domestica[†]	M domestica[‡]	G morsitans
<19	1 5	—	—	—
19	0 5	—	—	—
20	1 0	—	—	—
21	1 5	—	—	—
22	1 5	—	—	—
23	11 0	20 8	11 0	—
24	2 0	—	—	0 4
25	14 0	7 5	2 0	0 8
26	2 5	—	3 0	0 4
27	40 5	38 9	18 0	0 8
28	4 5	4 9	—	0 4
29	9 0	5 0	45 0	1 1
30	3 5	15 1	—	2 9
31	3 5	3 1	18 0	27 0
32	1 0	3 6	—	1 8
33	1 5	3 2	3 0	1 1
34	0 5	—	—	1 4
35	—	—	—	0 6
36	—	—	—	10 1
37	—	—	—	7 3
38	—	—	—	33 0
39	—	—	—	6 5
40	—	—	—	4 4

*Louloudes *et al*[10], laboratory strain
†Mixed flies collected Florida, July 1973
‡Silhacek *et al*[5], Cradson-P unmated ♀

Table 1 Mating responses of mature adult male *G morsitans* to decoys consisting of dead flies receiving various treatments *post mortem*

Decoy	Treatment	No tested	Attempted copulations (%)	Successful copulations (%)
♂	Dead 0–24 h	71	0	0
♀	Dead 0–24 h 24–120 h at − 10 °C	222	90	79
♀	Vacuum dried	51	88	76
♀	Washed in (3 ml per fly)			
	Hexane	60	0	0
	Benzene	20	0	0
	Chloroform	37	0	0
	Ether	247	11	6
	Ethanol	80	20	15
	Acetone	10	70	50
	10% Ethanol or acetone in H_2O	20	100	90

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Protection from habituation by lateral inhibition

HABITUATION, or response decrement, functions to reduce an animal's responsiveness to maintained or frequently repeated stimuli, while ensuring high responsiveness to novel stimuli Under some circumstances, however, habituation is inappropriate, in response to sensory afference caused by an animal's own activities, for example Mechanisms for protection from habituation under such circumstances are likely to be common Here we show, in locust movement detector (MD) neurones, that whereas habituation is rapid in response to repeated small field movements it is absent in response to maintained whole field movements Prolonged whole field movements do not therefore reduce the responsiveness of the MD neurones to subsequent small field movements Protection from habituation is achieved by lateral inhibition which acts prior to the site of response decrement to suppress the response to whole field stimuli

Part of the neuronal circuitry which mediates visually
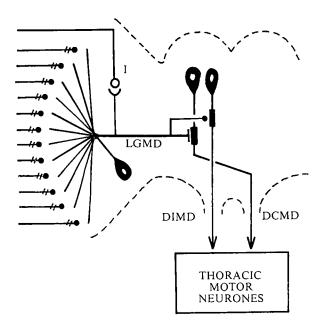
Fig 1 Highly diagrammatic representation of the anatomical relationships of the MD system The LGMD has a fan-shaped arborisation in the lobula of the optic lobe Its spikes transmit along its axon into the brain, where the neurone forms an electrical synapse with the DCMD neurone and an excitatory chemical synapse with the DIMD neurone Both these neurones run to the thoracic ganglia and make synaptic contact with motor neurones involved in jumping The main fan of the LGMD is the site of afferent synapses from the medulla, which supply a processed retinal projection to the LGMD The excitatory chemical synapses are markedly labile and habituate readily (indicated by double cross-hatching) The LGMD also receives, nearer the site of initiation of spikes, an inhibitory input (I) which derives its input from spatial summation over the whole of the visual field of the eye, and which is responsible for post-convergence inhibition in the system

induced escape jumping in the locust has been elucidated by intracellular recordings from identified interneurones and motoneurones[1-4] Three bilaterally paired MD neurones respond vigorously to abrupt movement of small and contrasting objects anywhere in the visual field They are, however, almost insensitive to whole field movements[5-7] The lobular giant movement detector (LGMD) of the optic lobe provides the main input to two cerebral units, the descending contralateral movement detector (DCMD) and the descending ipsilateral movement detector (DIMD) which relay spikes from the brain to the metathoracic motoneurones responsible for jumping The anatomy and major connections of the MD system are summarised diagrammatically in Fig 1

The LGMD is most responsive to new movement and derives this ability from rapid decrement of the response (habituation) produced when the same part of the retina is repetitively stimulated[6,8] As decrement is limited to the stimulated area, and as the fan-like arborisation of the LGMD is the site of convergence of the afferent pathways, habituation must occur presynaptically to it This is confirmed by intracellular recordings from the LGMD, which suggest that the habituation occurs presynaptically at the afferent synapses on the LGMD fan
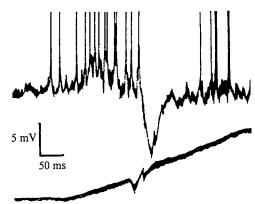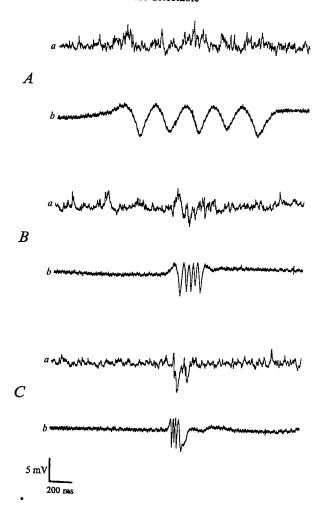
Fig. 2 Postconvergence inhibition in the LGMD of the response to a small moving target, produced by rapid displacement of the whole visual field The upper trace is an intracellular record (scale 100 ms and 5 mV) from the fan of the LGMD The slow ramp in the lower trace is the voltage analogue of the movement across the visual field of a small (about 10°) black target, which produces a large barrage of EPSPs and spikes in the LGMD The background consists of a stationary array of vertical black and white stripes covering the whole visual field About halfway through the response, the background is suddenly moved laterally, at a speed corresponding to 80 light–dark or dark–light transitions at the individual retinal receptor per second, for a period of 50 ms This is indicated by inflections on the ramp The movement of the background produces a strong hyperpolarisation which suppresses the response to small-field movement

Although there is little or no response to whole field movement the illumination of most or all of the ommatidia of the retina changes under such circumstances, depending on the complexity of the visual environment Palka[8] showed that large or whole field stimuli evoked both excitation and, preponderantly, inhibition in the MD system He found that the inhibitory effect of such a stimulus to one retinal area affected the response of the other areas equally, and not in proportion to their remoteness, as would be expected of a lateral inhibitory network We have corroborated Palka's results, and by intracellular recording shown the basis of the inhibition to be large summating inhibitory postsynaptic potentials (IPSPs) in the LGMD, which occur in response to rapid changes of illumination affecting large areas of the retina (Fig 2) That this type of inhibition acts proximally to the site of convergence is consistent with the observation that its effect is equal over the whole visual field
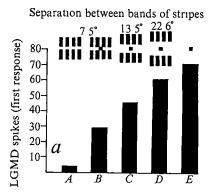
If this postconvergence inhibition was the only mechanism suppressing the response to whole field stimuli, all labile

synapses in the afferent pathways preceding the LGMD would be excited by such stimuli, and would habituate This would render the MD system blind to small field movements which followed soon after the cessation of whole field movement, and would clearly be maladaptive But in our experiments only rapid whole field stimuli produced postconvergence inhibition in the MD system (Fig 3) Slow whole field movements produced few excitatory postsynaptic potentials (EPSPs) in the LGMD and did not appreciably decrease responsiveness to a subsequent small field stimulus, showing that such movements do not excite labile synapses in the pathways to the LGMD There must, therefore, be a further inhibitory mechanism, which also suppresses the response to whole field stimuli, located peripherally in the optic lobe prior to the site of decrement We demonstrated this inhibition (Fig 4a), it suppressed the response to a small moving target, normally a very potent stimulus, and its effect was graded in proportion to the proximity of the large stimulating field to the small target The dependence on spatial separation is typical of a classical lateral inhibitory network, in which the components of an array of similar afferent channels are mutually inhibitory in inverse proportion to their spatial separation Figure 4b

shows that the lateral inhibition generated by a large moving stimulus not only largely suppressed the response to a simultaneously presented small target but also greatly reduced the rate and extent of habituation in response to the repeated movement of the small target The dynamics of this lateral inhibition were such that during rapid whole field movements some excitation reached the LGMD, where EPSPs were generated, the peripheral lateral inhibition was supplemented by postconvergence inhibition acting in the LGMD Such stimuli sometimes produce appreciable EPSPs in the LGMD, but these are prevented from initiating spikes by the summated IPSPs

Fig. 3 Postconvergence inhibition is produced only by rapid movements of large field stimuli The entire field of the eye is occupied by a vertical array of black and white stripes The upper trace (a) of each pair of records is an intracellular recording from the fan of the LGMD The lower trace (b) of each pair is the output from a photocell which is also illuminated by the same stripes as produce the field In A–C stripes are moved laterally across the visual field at velocities corresponding to 8, 20, and 70 light–dark or dark–light transitions per second at the retinal receptor All speeds of movement tend to produce an increase in EPSPs, the faster movements increasingly produce large IPSPs as well At eight transitions per second, no IPSPs are detectable

Fig 4 Lateral inhibition of the response to a small target a, The lateral movement of two bands of vertical stripes, which extend horizontally across the visual field, at a speed corresponding to seven transitions per second at the retinal cell, produces very few spikes in the LGMD (A) This speed of movement produces no postconvergence inhibition (compare Fig 3) When a small black target is added to the stripes, the response is appreciably increased The size of the response is a function of the proximity of the striped pattern to the small target (B–D) and the full response to the target is not seen until the stripes are totally removed (E) Trials were made at 2-min intervals in the order A–E, any habituation effects would therefore tend to counteract, rather than reinforce, the trend shown here The dimensions on the diagram are in terms of the angle subtended at the eye The two bands of stripes each subtend 22 6° vertically, and the period of the striped pattern is 15° The small target subtends 7 5° b, Lateral inhibition takes place more peripherally than habituation, and protects the labile synapses from depression during whole field movement The response to repeated movements (interstimulus interval = 10 s) of the small target alone shows a rapid decrement from its original high level (○) When a large area of moving stripes is added to the stimulus in close proximity to the target, however, the response is not only depressed (see A) but also shows no significant decrement (×) Both curves are normalised for comparison

Lateral inhibition is widespread in sensory systems of both vertebrates and invertebrates, and various functions have been suggested for it, such as contrast enhancement and an increase in the information content per action potential[9,10] In the MD system it has two other functions First by preventing a response to whole or large field stimuli, it is the major factor in determining that the MD system responds preferentially to small-field stimuli and ignores visual displacements generated by the animal's own movements Second, by acting prior to the site of decrement it protects the labile synapses from habituation, and therefore preserves the novelty detecting properties of the LGMD

We believe protection from habituation to be a new and possibly general role for lateral inhibition, and it may prove to be important in other than visual systems Protection from habituation is almost certainly a common device, because of the necessity of distinguishing sensory afference caused by the animal's own activities from that which is exogenous For example, tactile afferent pathways are protected during the escape tailflip of the crayfish by an efferent control network derived from the motor output system which produces pre-synaptic inhibition[11,12] The protection mechanism we describe is derived from the afferent pathways and has the added property of being independent of the motor system, it therefore also operates in the stationary animal This type of protection may also operate in the crayfish through inhibition generated by tactile afference (J J Wine and D Kennedy, personal communication) Indeed, wherever an interneurone is found with a wide receptive field and a preference for localised and novel stimulation within that field, lateral inhibition between the pathways which feed it may be expected

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Inverse relationship between serum IgG concentrations and measures of intelligence in elderly persons

WE have found a negative correlation between intelligence test scores and serum immunoglobulin concentrations in healthy subjects aged 45 yr and older This finding adds a potentially important dimension in studies of older persons who exhibit declining intellectual function

A longitudinal study of ageing in 268 elderly healthy volunteers was initiated in 1956 by the Duke University Center for the Study of Aging and Human Development The composition of this subject sample was proportionate to the race, sex and socioeconomic composition of similarly aged persons in the community from which they were recruited (Durham, North Carolina) The Weschler adult intelligence scale (WAIS) test was administered at the time of each visit to the centre Assessment of immune function was begun in 1967 with measurements of serum concentrations of IgG, IgA and IgM The method and reliability of these measurements has been detailed before[1]

Retrospective evaluation in 1973 revealed contemporaneous serum immunoglobulin levels and WAIS test scores for 97 persons (sample 1) Where more than one set of comparisons was available on any subject, the earliest comparison was selected for analysis Sample 1 (mean age$\pm$s e m $= 79\ 3\pm$ 0 5 yr, range 70–94) had a race and sex composition similar to the entire subject sample but had a higher mean WAIS test score Both raw and scaled WAIS scores were evaluated, the results presented were independent of the type of score used Serum immunoglobulin levels were $\log_e$ transformed before analysis, in normal populations the $\log_e$ values have a Gaussian distribution[1]
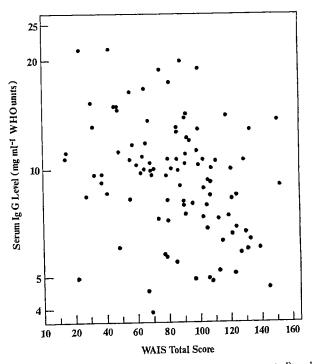
Fig 1 Scattergram of WAIS total score (not age-corrected) and serum IgG level in 97 subjects from the Duke Longitudinal Study ($r = -0\ 332, P < 0\ 005$)

Evaluation of sample 1 revealed a significant negative correlation between serum IgG levels and WAIS scores ($r = -0\ 332$, $P < 0\ 005$) (Fig 1), correlations for serum IgA ($r = 0\ 161$) and IgM ($r = 0\ 085$) were not significant (Table 1) This inverse relationship between the WAIS test and IgG was detected in all race and sex subgroups Although only four subtests are presented in Table 1, all 11 WAIS subtests scores were inversely related to serum IgG level In sample 1, WAIS score and age were correlated $-0\ 157$, IgG and age, were correlated $0\ 110$ To evaluate the relationship between the WAIS scores and serum IgG levels unencumbered by the effects of age, race and sex, a stepwise multiple regression analysis was performed with total WAIS score as the dependent variable Even after age, race and sex were forced into the regression equation, IgG accounted for a significant portion of the WAIS variability (partial $r = -0\ 296$, increase in multiple $R^2 = 0\ 082$, $P < 0\ 001$) To exclude the possibility that other sources of variation, such as the effects of repeated WAIS testing, disease and terminal illness, might account for the inverse relationship, regression analysis was also performed with the initial test scores of subjects who had no clinical evidence of illness and did not die within 2 yr of the IgG determination ($n = 67$, mean age $= 66\ 8\pm0\ 6$ yr) The IgG level still made a significant contribution to the regression equation (partial $r = -0\ 323$, increase in multiple $R^2 = 0\ 104$, $P < 0\ 005$) This suggests the observed relationship was not dependent on training effects or health status

The negative relationship was reproducible A second

Table 1 Correlations between WAIS scores and serum immuno-globulin level in two samples of older persons

WAIS subtests[‡]	Sample 1*			Sample 2[†]		
	IgG	IgA	IgM	IgG	IgA	IgM
Information	−0 363	0 183	0 061	0 018	0 006	0 006
Vocabulary	−0 319	0 120	0 065	−0 081	−0 065	−0 016
Digit symbol	−0 413	0 059	0 165	−0 098	−0 150	0 025
Picture arrangement	−0 217	0 152	0 045	−0 096	−0 100	0 026
Average scaled subtests§	—	—	—	−0 109	−0 121	0 027
Total WAIS score¶	−0 322	0 161	0 085	—	—	—

* $n = 97$, mean age$\pm$s e m $= 79\ 3\pm0\ 5$ yr
† $n = 468$, mean age$\pm$s e m $= 58\ 5\pm0\ 3$ yr
‡ All 11 WAIS subtests were administered to sample 1, but only the four in the table were administered to sample 2 The four subtests were selected because the previous study had demonstrated that the subtests, information and vocabulary, showed the most stability with increasing age, and the subtests, digit symbol and picture arrangement showed the greatest decline The seven subtests administered to sample 1 but omitted from this table were all negatively correlated with serum IgG level
§ Because an overall value such as total WAIS score was not available for sample 2, the average of the scaled scores for the four subtests was used
¶ This score was not age-corrected

longitudinal study was initiated at Duke University in 1967 consisting of 502 white persons aged 46–71 yr These subjects represented a broad range of the middle and upper socio-economic classes of Durham On the basis of the experience with the first longitudinal study, only four WAIS subtests were administered Information and vocabulary subtests were selected because they had shown the least change with age Digit symbol and picture arrangement were selected because they had shown the greatest change with age Sample 2 consisted of 468 persons (mean age $= 58\ 5\pm0\ 3$) on whom both WAIS scores and immunoglobulin levels were available from the initial round of testing In this younger, racially homogeneous sample, the correlation between IgG level and an average of the four scaled subtest scores was negative ($r = −0\ 109$, $P<0\ 02$) Seven out of ten (sex-by-5 yr age) subgroups in sample 2 showed the same inverse relationship Three of the four subtests exhibited a negative correlation with serum IgG level (Table 1) A similar relationship was detected in subject sample 2 between serum IgA and WAIS scores (Table 1) After deletion of age and sex effects, stepwise multiple regression analyses revealed that either IgG or IgA level made a significant, but not independent, contribution to the regression equation predicting each subject's averaged scaled subtest scores (for IgG, partial $r = −0\ 086$, increase in multiple $R^2 = 0\ 012$, $P<0\ 025$) This association between serum IgA and WAIS test scores could be the result of the relationship between IgG and IgA previously reported in younger persons[2] IgG and IgA were significantly correlated in sample 2 ($r = 0\ 26$, $P<0\ 001$), but not in the older sample 1 ($r = 0\ 13$)

The variability in WAIS test scores associated with IgG levels in both samples was small but significant The magnitude of this association could be a consequence of many other sources of variation, alternatively it may reflect a major influence present in only some members of each sample Although a precise mechanism cannot be identified from our data, the sevenfold increase in variability accounted for in the older subject sample is consistent with some consequence of biological age Several testable ageing-dependent hypotheses can be advanced to explain the observed relationship First, anti-brain antibodies increase with age[3] and might have a pathogenic role in cognitive decline Second, age-related decline in a third physiological system might account for decreased intellectual performance and elevated IgG levels Finally, an effect of selective mortality cannot be excluded Two lines of evidence support this last possibility First, individuals in both samples who died within 5 yr of the time of measurement (27 subjects in sample 1 and 28 subjects in sample 2) had lower WAIS scores and IgG levels than their surviving cohorts Second,

patients with senile dementia, which is associated with early death[4], have significantly lower serum IgG levels than age matched controls (R Green and C E Buckley, unpublished) A shortened life expectancy in persons with low IgG levels and low WAIS scores could contribute to the inverse relationship between measures of immune and intellectual performance detected in older persons

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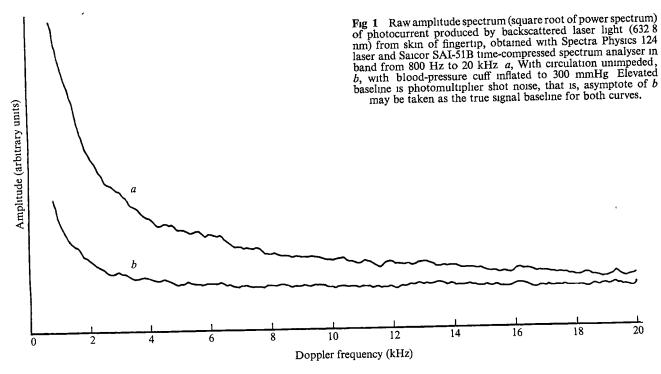
In vivo evaluation of microcirculation by coherent light scattering

THE microcirculation plays a central role in the regulation of the metabolic, haemodynamic and thermal state of the individual Its physiological state varies over both long and short time periods and reflects the state of health of the individual, in particular it is the ultimate arbiter of the adequacy of tissue perfusion in the presence of vascular disease or injury For these reasons it is of interest to both the physiologist and the clinician to be able to monitor the state of flow and distribution of flow in the compartments of the microvasculature rapidly, conveniently and with minimal interference with the many local and central reflex controls At present such measurements depend on direct observation, plethysmography thermal or radioisotope techniques[1], which are slow or cumbersome or grossly disturb the normal state of the subject We report here the first stages in development of a noninvasive method for acquiring such information which has a rapid response and the potential ability to distinguish flow in the different microvascular compartments by the differences in flow velocities

When coherent light is scattered from a complex medium which is in internal motion, the spectral line is broadened by the Doppler effect in a manner characteristic of the velocity distribution of the motion By heterodyning or homodyning the light on a square-law photodetector, this bandwidth may be brought to audio frequencies and examined with an electronic spectrum analyser[2] Information so obtained has been used in biology for measurement of diffusion coefficients of macromolecules[3], motility of bacteria[4,5], blood flow in retinal arteries[6] and viability of sperm[7]

Although the vascular bed of, for example, the skin is "visible" from the surface, in the sense that it imparts colour to the skin, light which reaches the blood and returns will generally suffer multiple scatterings during its diffusion through tissues At present this precludes a quantitative theoretical calculation of the Doppler spectrum, which must instead be calibrated empirically against conventional physiological measures, using the predictions of idealised theoretical models as a guide

As an example of the latter, we might imagine that photons wander randomly through densely diffusing stationary tissues, encountering one or more red blood cells moving in random directions with a speed distribution $U(v)$ The distribution $P_1(\Omega)$ of Doppler shifts of those photons which encounter one

Fig 1 Raw amplitude spectrum (square root of power spectrum) of photocurrent produced by backscattered laser light (632 8 nm) from skin of fingertip, obtained with Spectra Physics 124 laser and Saicor SAI-51B time-compressed spectrum analyser in band from 800 Hz to 20 kHz *a*, With circulation unimpeded, *b*, with blood-pressure cuff inflated to 300 mmHg Elevated baseline is photomultiplier shot noise, that is, asymptote of *b* may be taken as the true signal baseline for both curves.

red cell may be found from the basic Doppler formula $\Omega = K.v$ by averaging over all the random directions involved, after some manipulation of integral identities it may be shown that

$$v^2(d^2P_1(2v/\lambda))/dv^2 = \lambda U(v)/2$$

where λ is the wavelength of the light The higher order multiple red cell encounters, P_n (Ω) are the self-convolutions of P_1 (Ω), since the photon suffers the sum of a number of independent random Doppler shifts From arguments of this sort it may be shown that (1) in principle it is possible to recover the cell speed distribution $U(v)$ from the measured spectrum, (2) the spectrum is expected to be a monotonically decreasing function of frequency, higher frequencies corresponding to faster flow speeds, and possibly possessing an integrable singularity at $\Omega = 0$, (3) the scattered light will be depolarised, with two statistically independent polarisation components each having Gaussian statistics, (4) for a random flow field with an r m s velocity of 1 cm s^{-1} the r m s bandwidth of 632 8 nm light will be 12,903 Hz times the square root of the mean number of red cell encounters (plus small corrections for the shape of red cells and refractive index)

These conclusions are, of course, based on idealised assump-

Fig 2 Power spectrum of Doppler flow signal from fingertip at intervals following ingestion of 30 ml of ethanol Arbitrary units Bandpass 5–50 kHz Data smoothed by neighbouring point averaging corrected for shot noise and bandpass filter response *a*, Control before ethanol administration, *b*, *c* and *d* follow ingestion by 5, 10 and 15 min

tions and should be taken only as a guide to the interpretation of empirical spectra

We have undertaken preliminary experiments to determine the feasibility of this method Light from a 15 mW HeNe laser illuminated a 1 mm area of skin of a fingertip The backscattered light was sensed through a pinhole by a photomultiplier The homodyne photocurrent which resulted was passed through a variable bandpass filter to a time compression digital spectrum analyser To evaluate the possibility of following rapidly the dynamics of the circulation, the signal within a particular passband was rectified and displayed on a chart recorder as a function of time

Figure 1 shows the spectrum before and after occlusion of the brachial artery with a blood-pressure cuff, demonstrating that the Doppler signal is indeed caused by blood flow The bulk of the flow signal power is actually in the band below 1 kHz but that region is more subject to artefacts resulting from tissue motion in the crude preliminary apparatus

To demonstrate the feasibility of monitoring pharmacological effects on the microvasculature, a known vasodilator, in this case a small amount of ethanol, was administered Figure 2 shows successive spectra The frequency band shown corresponds to speeds found only in arterioles, venules, and shunts The result is consistent with increased flow in large vessels and increased maximal flow velocity

Figure 3 shows the variation over time of the signal power in the 10–12 kHz band as a blood-pressure cuff is rapidly inflated above the systolic pressure and then slowly let down The initial decay is the time required for large arteries to empty before the microvascular flow stagnates When the cuff is held between systolic and diastolic pressures until the veins have filled, the flow becomes highly pulsatile In this time-resolved mode it was observed that fingertip flow is quite labile in response to room temperature, posture, respiratory pattern and emotional stimulation of the subject We know of no comparable method of demonstrating these rapid microvascular reflexes

Laser Doppler spectroscopy therefore offers a promising tool for examination of the physiological state of the microcirculation rapidly and non-invasively Because it provides information about the flow velocity distribution, it has the potential ability to analyse the distribution of flow in the different microvascular compartments Applications in vascular physiology, clinical evaluation of vascular disease and assessment of the viability of burns and grafts are foreseen We would like to thank Dr Ralph
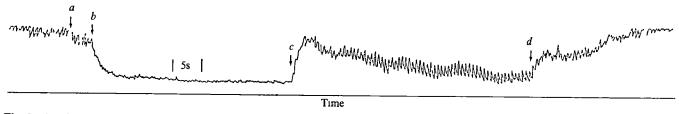
Fig. 3 Amplitude of Doppler flow signal (including shot noise baseline) in the band 10–12 kHz as a function of time during inflation of a blood-pressure cuff followed by its slow release Point *a* is beginning of inflation At *b* the cuff exceeds the systolic pressure Cuff pressure was held above systolic for 20 s and then slowly released At *c* the cuff pressure dropped below systolic, at *d* cuff pressure fell below diastolic By the end of the chart cuff pressure was entirely released Amplitude scale arbitrary

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Alterations in behaviour and catecholamine biosynthesis induced by lithium

THERE IS general agreement that lithium (Li) impairs central catecholamine transmission, although the underlying mechanism is subject to debate[1–4] More ambiguous is the literature describing the behavioural effects of Li in experimental animals Although considerable evidence indicates that Li suppresses various behaviours[5–10], there are reports that this effect is only apparent after drug-induced stimulation[11–14] Results are also contradictory with respect to the behavioural effects of long term Li administration, some studies reporting continued or even exaggerated suppression with repeated administration[15–18], while others describe development of tolerance to the Li-induced behavioural suppression[12,13]

Lithium is well established as a treatment for mania and a prophylactic agent for prevention of recurrent mood disorders[19–21] Thus, the elucidation of its behavioural and neurochemical effects may provide some insight into the aetiology of naturally occurring mood disorders Since some degree of long term treatment with Li is, however, usually required before clinical improvement is apparent, it is essential to evaluate potential clinically relevant effects in chronically treated animals In our study of the behavioural and neurochemical correlates of long term Li administration, we have found that Li suppresses both spontaneous and amphetamine-induced behaviour in rats, that tolerance develops to this suppression, and that the emergence of tolerance is associated with increased tyrosine hydroxylase activity in the substantia nigra and caudate-putamen of rats

Male rats (350–375 g, Carworth Farms) were housed in groups of four and maintained on a 12-h light–dark cycle and allowed free access to food and water Locomotion was monitored automatically in sound-attenuated chambers[22,23]

All rats received nine daily injections, the last immediately before testing in the chambers The group on a regime of long term treatment received eight daily injections of LiCl (1 5 meq kg⁻¹) and a single injection of saline before testing Two

acute groups were injected with Li, either 24 h or immediately before testing The control group received nine injections of saline. Injections were given subcutaneously at the same time each day in volumes of approximately 3 ml in order to minimise local irritation produced by Li

After the final pretreatment injections, animals were placed in the chambers and their spontaneous activity was monitored for 2 h Then, all groups received an injection of *d*-amphetamine sulphate (0 5 mg kg⁻¹, intraperitoneally as free base) Behaviour was monitored for an additional 2 h, at which time the behavioural response to this dose of amphetamine had subsided[22,23]
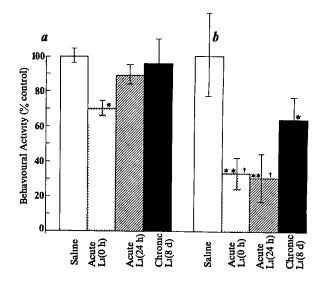
Fig. 1 Effect of acute and chronic Li administration on spontaneous locomotor activity (*a*) and on *d*-amphetamine-induced behavioural excitation (*b*) Single injection of Li (1 5 meq kg⁻¹, subcutaneously) only reduced spontaneous activity in the 0-h acute group Excitation produced by amphetamine (0 5 mg kg⁻¹, intraperitoneally), however, was depressed in both acute groups and, to a lesser extent, in the 8-d Li group
*Significantly less than saline pretreated controls, *P* < 0 02
†Significantly less than chronic Li group, *P* < 0 05
**Significantly less than saline pretreated controls, *P* < 0 001

During the first 30 min after initial exposure in the chambers, only the 0-h acute group showed a decrease in activity compared with saline controls (*P* < 0 02, Fig 1) During the second-hour interval before injection of amphetamine, all groups remained relatively inactive Injection of amphetamine produced a moderate increase in locomotion for the first 30 min after injection (approximately 100 cross-overs for the saline pretreated group) Acute pretreatment with Li significantly suppressed the amphetamine-induced activity to 30–40% of saline control levels (*P* < 0 01) for up to 24 h after injection of Li Tolerance to the suppressive effects, however, seems to have occurred by 8 d, for the animals repeatedly injected with Li exhibited significantly more amphetamine-induced activity than did either of the acute groups (*P* < 0 05), but less than that of the saline control group (*P* < 0 02)
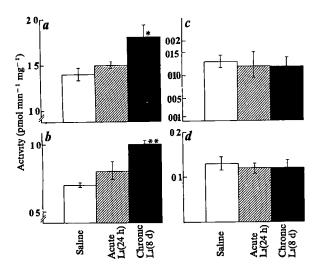
Fig. 2 Effect of acute and chronic Li administrationon tyrosine hydroxylase activity in the cell body and nerve ending regions of the nigro-striatal pathway (substantia nigra (b) and caudate-putamen (a), respectively) and in the dorsal noradrenaline pathway (locus coeruleus (d) hippocampus-cortex (c), respectively) Eight-day Li treatment resulted in an augmentation in tyrosine hydroxylase activity only in the substantia nigra and caudate-putamen

*P < 0 05
†P < 0 001

To examine the possible neurochemical correlates for these behavioural effects, rats comparable with those used for the behavioural experiments were injected with either saline or Li for 8 d, or with saline for 7 d and with Li on the eighth day All rats were decapitated 24 h after the last injection, their brains were removed quickly and four discrete regions were dissected using a brain slicing device[24] The regions were homogenised individually and examined for tyrosine hydroxylase activity by a modification of the Nagatsu procedure[25,26] The areas examined included the cell body and nerve ending regions of the dopaminergic nigro-striatal pathway (substantia nigra and caudate-putamen, respectively), and the cell body and nerve ending regions of the dorsal noradrenergic pathway (locus coeruleus and hippocampus-cortex, respectively)

Alterations in tyrosine hydroxylase activity were found only in the dopaminergic system (Fig 2) Acute Li produced a slight although insignificant increase in tyrosine hydroxylase activity But 24 h after eight daily injections of Li, the enzyme activity in both substantia nigra and caudate-putamen had increased substantially (30 and 40 % greater than corresponding controls), thus suggesting that the nigro-striatal dopamine pathway plays a role in the development of tolerance to the behavioural suppression induced by Li That is, an increased availability of dopamine, consequent to the elevation of tyrosine hydroxylase activity, might partially overcome the impaired transmission produced by Li

The failure to obtain enzyme changes in the noradrenaline regions examined seems to conflict with other reports of Li-induced increases in the turnover rate of brain noradrenaline[16,27-29] Most of these results are, however, based on whole brain assessment of turnover, while our study was restricted to the dorsal pathway It is conceivable, therefore, that the Li-induced alterations in turnover of noradrenaline are limited to the ventral or other brain noradrenaline systems The lack of a response in the dorsal noradrenaline pathway does not indicate that in this system tyrosine hydroxylase activity is refractory to change, since we have found that either chronic reserpine or desmethylimipramine administration produces marked alterations in the activity of tyrosine hydroxylase in the locus coeruleus and hippocampus-cortex[30]

With respect to brain dopamine pathways, Ho *et al*[31] report no alterations in turnover in four brain regions after several

weeks of Li treatment. Friedman and Gershon[32], however, found a substantial decrease in dopamine biosynthesis in striatal brain slices after 14 daily injections of Li Several differences in procedure may account for the discrepancy between our results and those of Friedman and Gershon, not the least of which is the large difference in time between last injection and biochemical determination (that is, 1 h compared with 24 h)

Whether or not continued chronic Li treatment would result in complete tolerance to its suppressive effects remains to be determined; however, if complete tolerance is found to occur, then the action of Li on amphetamine-induced behavioural hyperactivity would seem to be an inappropriate model for its clinical effects Irrespective of the clinical implications, however, the neurochemical events underlying the tolerance development to Li-induced behavioural suppression are of considerable theoretical interest We have shown before that prolonged impairment of central catecholamine transmission, produced by drugs or other treatments, results in an increase of tyrosine hydroxylase activity[33-35] The converse relationship is obtained when synaptic transmission is facilitated for relatively long periods[30] Thus, our finding that an augmentation of tyrosine hydroxylase activity is produced by Li is consistent with these findings Such an increase in catecholamine biosynthetic capacity may represent a compensatory response to the Li-induced impairment of catecholamine transmission which partially counteracts the suppressive effects of Li on behaviour

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Lipopolysaccharide induces C-type virus in short term cultures of BALB/c spleen cells

In certain strains of mice, such as AKR, C-type viruses occur throughout life and can readily be detected by infectivity assays[1,2]. In other seemingly virus-free strains, C-type viruses can be activated *in vivo* by X irradiation[3,4], chemical carcinogens[5], graft-*versus*-host reactions[6] and *in vitro* by long term cultivation[7], by X rays[8], halogenated pyrimidine analogues[9,10] and mixed lymphocyte reactions[6]. We report here that lipopolysaccharide, a B-cell mitogen[18], induces C-type virus in short term cultures of BALB/c spleen cells

During the first 24 h, spleen cell cultures from 6-8 week old male BALB/c mice (Bomholdgard, Denmark) contained lipopolysaccharide W, *E coli* 0111 B4 (LPS, Difco, 16 µg ml⁻¹) or concanavalin A (con A, Calbiochem, 4 µg ml⁻¹) or phytohaemagglutinin-P (PHA, Difco, 10 µg ml⁻¹) or no mitogen Pellets obtained from the pooled day 3 and day 4 supernatants of four groups (LPS, con A, PHA and control), each originating from 50 cultures (1 ml) were assayed for reverse transcriptase activity[12] with poly(A)ₙ·poly(dT)₁₂₋₁₈ as template primer Figure 1 shows that LPS-treated culture supernatants contained enzyme activity which was linear for about 60 min, while in control cultures or in con A- and PHA-stimulated cultures no activity was found Enzyme induction could be reproduced using a highly purified LPS from *Salmonella abortus equi*

To characterise further this activity, 25 combined LPS-treated 1-ml cultures were analysed following isopycnic centrifugation on a 15-60% sucrose gradient (Fig 2) A peak of activity was
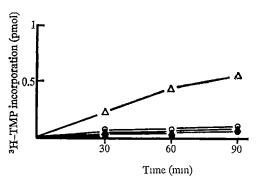
Fig. 1 Reverse transcriptase assay in samples prepared from concentrated supernatants from cell cultures stimulated with LPS (△), con A (▲), PHA (●), or control (○) Reaction mixtures (100 µl) contained 20 µl sample, 40 mM Tris, pH 7 9, 60 mM KCl, 1 mM dithiothreitol, 1 5 mM Mn(II)-acetate, 0 2 mM EDTA, 0 1% Nonidet P-40, 20 µg ml⁻¹ poly(A)ₙ poly(dT)₁₂₋₁₈ (poly(A)ₙ (Miles) and dT (Collaborative Research) were annealed in equimolar amounts), 10 µM ³H-TTP (Amersham, 7,500 d p m pmol⁻¹) Incubation at 30 °C was stopped at the indicated times by the addition of an excess of 10% trichloroacetic acid (TCA) containing 0 02 M sodium pyrophosphate Following 30 min incubation in ice, acid-precipitable radioactivity was washed on a GF/C glass fibre filter (Whatman) with cold 5% TCA and processed for liquid scintillation counting using standard procedures Reaction mixtures containing no virus were included for background determination which was subtracted from the experimental values Spleen cell cultures were prepared as described previously[11] A 1 ml culture contained 2 × 10⁶ viable nucleated cells in loosely capped disposable 12 × 75 mm tubes (Falcon) Incubation was at 37 °C in CO₂ in air (8%) Culture medium RPMI 1640 (Microbiological Associates) containing 8% foetal bovine serum (Rehatuin, Reheis), penicillin (50 IU ml⁻¹, Hoechst) and streptomycin (50 µg ml⁻¹, Novo) was changed every 24 h The removed medium from days 2-4 was centrifuged (1,000g for 10 min) and kept at −20 °C until further examination Then, 1 ml samples were pooled from corresponding cultures after thawing at 30 °C, centrifuged (35,000g for 60 min) at 4 °C and resuspended in 1/50 of the original volume in buffer (0 01 M Tris, 20 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 50% (v/v) glycerol, pH 7 9)

observed at 1 16 g cm⁻³, the density characteristic for C-type viruses

Some cellular enzymes are known to polymerise thymidine monophosphate using poly(A)ₙ poly(dT)₁₂₋₁₈ as template primer, but they work more efficiently with poly(dA)ₙ·poly(dT)₁₂₋₁₈ (refs 13-15) Moreover, terminal nucleotidyl transferase from thymus cells can be stimulated by dT₁₂₋₁₈ (ref 16) To distinguish whether the LPS-induced
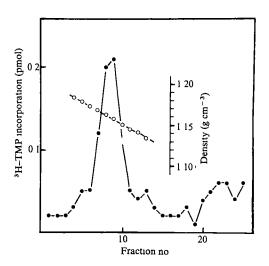
Fig. 2 Sucrose density gradient centrifugation A pellet prepared from pooled day 2 to 4 supernatants of LPS-stimulated cultures was resuspended in phosphate buffered saline, pH 7 4, layered over a 15-60% sucrose gradient and centrifuged in a Beckman SW65 rotor at 35,000 r p m for 16 h at 4 °C Three drop fractions were collected from the bottom and 50 µl per fraction were analysed for reverse transcriptase activity (90 min assay) For conditions see Fig 1 Density determinations of selected fractions were performed with an Abbé refractometer

enzyme activity represents C-type viral reverse transcriptase or cellular enzymes, poly(A)ₙ poly(dT)₁₂₋₁₈, poly(dA)ₙ poly (dT)₁₂₋₁₈ and poly(dT)₁₂₋₁₈ were compared for their capacity to stimulate the enzyme (Table 1) Sucrose gradient purified Rauscher leukaemia virus (RLV) and a spleen cell homogenate were included as controls LPS-induced activity as well as reverse transcriptase from RLV showed a strong preference for poly(A)ₙ poly(dT)₁₂₋₁₈ over poly(dA)ₙ poly(dT)₁₂₋₁₈ but no detectable activity with poly(dT)₁₂₋₁₈ The cellular enzymes preferred poly(dA)ₙ poly (dT)₁₂₋₁₈ and were also stimulated by poly(dT)₁₂₋₁₈ The presence of C-type particles in LPS-stimulated cultures was verified using electron microscopy

Table 1 Template-primer requirements for enzyme activity

Source of enzyme	Template-primer		
	Aₙ dT₁₂₋₁₈	dAₙ dT₁₂₋₁₈*	dT₁₂₋₁₈†
Supernatant of LPS-stimulated spleen cells‡	0 79	0 08	0 05
Rauscher leukaemia virus	9 87	0 06	<0 01
Supernatant of normal spleen cell homogenate§	0 14	0 83	0 07

Same assay conditions as described in Fig 1 Incubation time was 90 min Mean values of triplicate determinations are expressed in pmol ³H-thymidine-monophosphate incorporated per 20 µl concentrated supernatant

* poly(A)ₙ poly(dT)₁₂₋₁₈ of standard reaction mixture replaced by poly(dA)ₙ poly(dT)₁₂₋₁₈
† poly(A)ₙ poly(dT)₁₂₋₁₈ of standard reaction mixture replaced by poly(dT)₁₂₋₁₈
‡ Pooled supernatants harvested on days 3 and 4
§ BALB/c spleen cells were homogenised by hand in normal saline and cleared by centrifugation
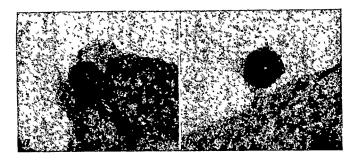
Fig 3 Electron micrographs of C-type virus particles in 3-day BALB/c mouse spleen cultures induced by LPS Sections from cells double-fixed in suspension with glutaraldehyde and osmium tetroxide ($\times$ 120,000) *a*, Budding particles, *b*, extracellular mature particles

(Fig 3) Typical budding as well as extracellular virus could be detected

It is surprising that an ubiquitous agent such as LPS, a constituent of Gram-negative bacteria, physiologically occurring in the intestinal flora, induces C-type virus Evidence that induction of endogenous C-type virus may be quite frequent *in vivo* was reported by Aaronson and Stephenson[17] who found that most mouse strains tested had high antibody titres against endogenous xenotropic virus The relationship between LPS-induced virus, endogenous mouse-tropic virus and xenotropic virus should be investigated further It will be interesting to see whether LPS also induces C-type viruses in other species, including man

Purified LPS from *Salmonella* was provided by Dr Ch Galanos, Max-Planck-Institut fur Immunobiologie, Freiburg I Br Dr E Suter carried out the electron microscopic study

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Sudden appearance of IgG plaque-forming cells

Experiments described here show that a large number of indirect, presumably IgG, plaque-forming cells (PFC) appear within a few hours during the initial stages of the IgG response of BALB/c mice to sheep red cells When washed spleen cells

are placed *in vitro* over the appropriate period indirect PFC develop as well as *in vivo*, but a few hours earlier

These experiments began when it was found that the count of indirect PFC (using the technique of Cunningham *et al.*[1]) rose from an undetectable level on the fourth day after immunisation (day 4), to the peak value (2×10^5 to 4×10^5 per spleen) by early on day 5 We followed events during day 4 in
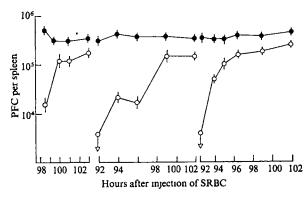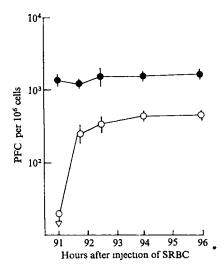
Fig. 1 Groups of 6 BALB/c female mice received 10^8 sheep red blood cells (SRBC) intravenously and PFC were assayed at intervals thereafter, by the method of Cunningham *et al*[1], but incubating for 30 min only Indirect PFC were developed by a rabbit serum against mouse IgG This was raised using purified mouse IgG and, on immunoelectrophoresis against whole mouse serum, showed only a single line, reacting with IgG It slightly inhibits PFC on day 2, when one expects the majority of PFC to be IgM (ref 3) and considerably increases the count from day 5 onwards when the majority of PFC are IgG The day-to-day levels of PFC developed by this serum follow closely those reported by other workers[2] Points are geometric means Bars indicate 1 s e ●, Direct PFC, ○, Indirect PFC, circle joined to triangle, value less than lowest level detectable (4,000 per spleen)

detail and, in repeated experiments, found that the count had risen to around 10^5 within 8–9 h When we followed the count at intervals of approximately 2 h, we found a sharp rise within the initial 2–4 h (Fig 1) These figures are means from groups of 6 mice and, as it is unlikely that the rises were simultaneous, the increase in an individual mouse could have been even more rapid

The same rise in IgG PFC was seen when washed spleen cells were incubated *in vitro* over the appropriate period The best

Fig 2 Spleens were removed 90 h after SRBC injection A pool of washed cells was prepared in RPMI 1640 containing 5% foetal calf serum 1 ml containing 3×10^7 nucleated cells was placed in each of several polystyrene tubes (12×75 mm), which were gassed with CO_2–air (5 95) and rotated on a blood cell suspension mixer at 37 °C At the times shown, 0 05 ml was removed for PFC assay, and the tubes regassed and replaced Tubes which had not been opened during the experiment gave the same counts at 96 h as those which had been sampled repeatedly

results were obtained in tubes rocked or rotated so that the cells remained in suspension in these conditions few cells became adherent One of these experiments is shown in Fig 2, during a 1 5-h incubation (2 5 h after spleen removal) a large number of IgG PFC appeared and the ratio of indirect to direct PFC is similar to what is found *in vivo* (compare Fig 1) Experiments of the type illustrated in Fig 3 show that populations placed in culture can develop IgG PFC earlier than they would *in vivo* For instance, population B, removed from mice at 86 h has developed 300 IgG PFC per 10^6 cells (equivalent to 3×10^4 or more per spleen) by 90 h, while population D, when removed from the same group of mice at 91 h had no demonstrable IgG PFC
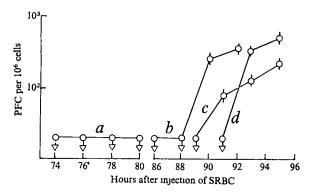
Fig. 3 As Fig 2 but cells removed at different times after injection of SRBC Only indirect (IgG) PFC are shown, to avoid complicating the figure Counts of direct (IgM) PFC varied no more than in Fig 2, and had the following mean values *a*, 334, *b*, 1,406, *c*, 957, *d*, 1,439 PFC per 16^6 cells

Our results suggest the following sequence of events in these mice Between 74 and 86 h the spleen develops the ability to generate rapidly a large number of IgG PFC The results obtained *in vitro* show that this process (the generation of IgG PFC) does not require a structured microenvironment, nor continued humoral influences, nor perhaps adherent cells From 86 h (when IgG PFC can appear *in vitro*), the process is suppressed *in vivo* until around 92 h when a large number of PFC appear suddenly

The development of large numbers of IgG PFC within periods of 12–24 h has been observed in several other strains of mice[2-4], hence the events we have described may occur more widely

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In vitro ageing and evolution of immunocompetence in long term normal lymphoid cell cultures

CULTURE of dissociated lymphoid cells *in vitro* enables the description of various characteristics of an immune response as manifested under defined conditions—but not the time course of evolution Normal lymphoid cell cultures are usually

short term, those of spleen or lymph node cells from common laboratory animals rarely surviving longer than 12–14 d Lymphoblastoid or plasmocytoma lines, established in continuous cultures originated from cancerous or Epstein-Barr virus-bearing tissues, seem to have lost their capacity for responding to specific antigens We have shown[1,2] that calf lymphoid cells can be maintained in more prolonged cultures (up to three months) without losing their immunocompetence We report results obtained using dissociated calf lymph node cell cultures surviving for more than 2 yr

Culture methods were as described previously[1] briefly, lymph nodes from 3–4-month-old animals (local slaughterhouse) within 5 min after slaughtering, were freed from surrounding tissue, quickly swabbed with ethanol, and brought to the laboratory in sterile culture medium at 37 °C After elimination of fat and outer connective tissue, the lymphoid tissue was cut into fine pieces The minced material was gently teased through a steel sieve into Eagle's MEM (Difco), buffered at pH 7 2 with sodium bicarbonate, and supplemented with non-essential amino acids without glutamine, colimycin 50 U ml^{-1}, and fungizone 10 μg ml^{-1} Normal calf serum was added at 5% concentration Aggregates were allowed to settle and the supernatant material was centrifuged at 800 r p m for 10 min The cells were then suspended in the above culture medium at a concentration varying from 8×10^6 to 2×10^7 ml^{-1}, and distributed in 1,000 ml blood transfusion flasks, each receiving 250 ml of cell suspension The culture flasks were incubated at 37 °C in a humid atmosphere of CO_2-O_2 (5 95) without agitation

Viability of the cells, estimated using trypan blue exclusion, in two different cultures is shown in Fig 1 About 60% of cells died during the first 2–4 months of culture, after this initial period, the viability did not seem to be greatly altered for more than 1 yr During these long term cultures, only moderate changes in size distribution of the cells were observed Thus in three 'blind' examinations, we found a slight but reproducible variation in the relative percentages of cells of 5–9 μm and 10–15 μm in diameter The latter represented 10% of the total viable cell population at day 1 of the culture, but only 5% at day 735 Thus the vast majority of the calf lymph node cells consisted of small and medium sized lymphocytes

We studied the ability of the lymphoid cell suspensions to respond either to a non-specific mitogen, phytohaemagglutinin (PHA) (Difco), or to specific antigens, sheep red blood cells (SRBC) and φ17 phage, active on *Streptomyces chrysomallus*, strain 17 (ref 2) With PHA, a moderate stimulation of DNA synthesis, measured by incorporation of ^3H-thymidine (2 μCi ml^{-1} of the culture medium containing 10^6 cells) was observed only during the first days of culture At the optimal time of incubation (63 h), and with the optimal dose (0 01% final concentration), the stimulation index, calculated as the ratio, (c p m in stimulated sample)/(c p m in control sample), did not exceed 2 8 It should be noted, however, that even in these young cultures the level of incorporation in the control cultures in the absence of PHA (500–600 c p m per 10^6 cells) was lower than that usually found in comparable cultures of mouse or rabbit lymph node cells

From day 50 to day 58 of culture, the response to PHA was measured after 48 h of contact with the mitogen As the incorporation in the control cultures without PHA was still lower than that found previously (less than 100 c p m per 10^6 viable cells), no enhancement of incorporation was observed after treatment with PHA The situation was the same at day 778

The capacity of the cultures for *in vitro* response to antigens was studied using two methods the formation of direct plaque forming cells (PFC) after stimulation with sheep red blood cells (SRBC), and the determination of the frequency of response units to φ17 phage[3] Stimulation with SRBC was performed using the above culture medium, containing 5×10^{-5} M 2-mercaptoethanol, and two RBCs per calf lymphoid cell
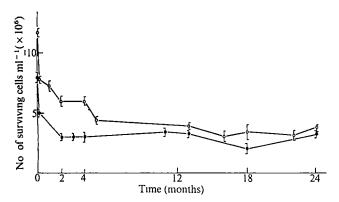
Fig. 1 Survival of calf lymph node cells in two different long term cultures as determined by trypan blue exclusion Means ± s e from five independent samplings Bars indicate calculated s e

The number of direct PFC was measured each day after stimulation, using the CMC method[4] No background of PFC was found in the absence of stimulation In culture to which antigen was added, the maximum level of response was reached at day 9 after stimulation Four independent determinations of the number of PFC were performed at each period of the culture

Using young cultures of calf lymph node cells (up to 2–3 weeks) the maximal number of PFC reached was 357±71 per 10^6 cells It was 342±45 per 10^6 cells after 7 months Thus, although the number of viable cells had decreased by more than 50%, the proportion of cells able to form plaques remained unchanged during this period By contrast, after 2 yr of culture, the number of PFC was only 154±47 (a young culture (1–3 weeks) was simultaneously studied and gave from 350 to 450 PFC per 10^6 cells), the situation throughout this period was reversed, compared with the previous one A 30% decrease in viability took place from day 21 to day 778, whereas a 50% decrease in number of PFC was observed for the same period, thus the proportion of PFC to total viable cells decreased by about 40%

This effect of ageing on the immunocompetence was confirmed by studying the frequency of response units towards φ17 phage The methods used for preparing and statistically analysing microcultures containing variable numbers of active lymphoid cells were as described previously[3] Briefly the main characteristic of the method is to preserve good survival of normal lymphoid cells by dilution into irreversibly inactivated cells of the same origin Inactivation without noticeable alteration of the viability was obtained by previous treatment with streptovitacin (U9361) This procedure permits the use of very small numbers of active lymphoid cells in optimal conditions to carry out a primary in vitro response These cultures are phage-stimulated and are capable of neutralising the phage by producing IgM antibodies The smaller the number of active cells in the microculture the greater the percentage of negative microcultures Positive microcultures are considered to contain at least one complete response unit, the frequency of which is calculated Four independent series of microcultures were carried out in each case Each series involved sixteen wells for each experimental point The results were as follows up to day 245, no noticeable variation in the frequency of response units (1 per 2,100–2,500 cells) was observed From day 723 to day 770 of culture, the frequency seemed to be considerably altered. In four different experiments we found a value of 1 response unit for 50,000–70,000 cells Such a reduction during a period of 16–17 months corresponds to the disappearance of more than 95% of cells able to produce antibodies neutralising significantly (more than 20%) φ17 phage

The various results reported here must be compared with the results obtained for in vivo experiments Price and Makinodan[5] have shown that an 80% decrease in the number of direct PFC was observed during the primary response of 30–32-month-old

mice, compared with 3-month-old mice Such a decrease was assigned mainly to the reduced rate, or capacity, of division of both the B and T cells Our in vitro experiments do not permit us to rule out this hypothesis, although the evolution of the in vitro immunocompetence does not seem to be directly related to that of the potentiality of proliferation Indeed, very few dividing cells seem to be present in the calf-lymph-node cell suspensions, even in the first days after removal from the animal After PHA stimulation, the level of DNA synthesis is low, however, the immunocompetence, estimated in terms of PFC or anti-phage response units, is high in the young cultures, and does not seem to vary for several months It is only after the first year of culture that the immunocompetence is affected

The evolution of immunocompetence thus seems to be independent of that of the proliferative capacity. The specificity of the immune response emphasises the particular significance of these results and supports the hypothesis that the process of ageing expresses intrinsic cellular deficiencies Moreover, the fact that the frequency of response units towards the phage is more altered than the response measured in number of PFC, suggests that the cells producing large amounts of antibody are relatively less affected by these intrinsic deficiencies Clearly, these experiments do not disprove the genetic mutation hypothesis of ageing[6] but rather lend support to some sort of cytoplasmic hypothesis, either Orgel's error catastrophe hypothesis[7], or the accumulation of cytoplasmic breakdown products[8] disturbing the mechanisms of antigen recognition and/or antibody biosynthesis

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Effect of a protein-free diet on lymph node and spleen cell response *in vivo* to blastogenic stimulants

A PROTEIN-deficient diet determines an early involution of the thymus, and the quantitative difference between lymphocytes from blood, lymph node and spleen of intact and of thymectomised rats is generally smaller with a protein deficient diet than with a normal one[1,2] On the other hand, the decrease in the size of blood lymphocytes[1], and in the viability[3] of lymphocytes from lymph node and spleen, following deprivation of dietary protein is conditioned to a large extent by the atrophy of the thymus

These data already militate in favour of a predominant depletion and functional inactivation of the thymus-dependent (T) lymphocytes in experimental protein malnutrition This conclusion is corroborated by the findings we report here, which show that a protein-free diet inhibits to a much greater extent the lymphocytic activation induced *in vivo* by blastogenic stimulants known to be almost exclusively (phytohaemagglutinin[4]) or partly (sheep red blood cells[5,6]) thymus-dependent than the

Table 1 Changes in body weights and weights of a popliteal lymph node and of the spleen in normally fed and protein-deprived rats on day 4 after subplantar injection of PHA, LPS and SRBC

Group	Normal diet Body weight (g)	Weight (mg) Popliteal lymph node	Spleen	Protein-free diet Body weight (g)	Weight (mg) Popliteal lymph node	Spleen
Non-injected controls	306 9 ± 3 8 (7)	7 8 ± 0 6	623 5 ± 31 7	113 6 ± 1 3 (18)	3 4 ± 0 4	138 6 ± 6 5
Injected with PHA	319 3 ± 8 0 (6)	19 9 ± 3 9	714 2 ± 28 5	112 3 ± 1 2 (12)	4 0 ± 0 5	136 6 ± 9 1
Ratio injected/ non-injected		2 55	1 14		1 19	0 98
Injected with LPS	322 0 ± 8 4 (6)	12 6 ± 1 3	709 0 ± 21 8	117 3 ± 1 5 (10)	3 6 ± 0 8	209 8 ± 14 4
Ratio injected/ non-injected		1 62	1 14		1 08	1 51
Injected with SRBC	301 8 ± 16 7 (6)	20 1 ± 2 3	663 5 ± 20 2	112 6 ± 1 6 (12)	6 0 ± 0 8	113 3 ± 8 3
Ratio injected/ non-injected		2 58	1 06		1 74	0 82

Values are means ± s e The numbers of rats are indicated in parentheses The weight changes in the organs due to the stimulants are represented by the ratios between injected and non-injected rats on the same diet

Composition of the salt mixture in protein free diet $CaCO_3$, 18 90, $CaHPO_4$ 2 H_2O, 12 60, KCl, 14 90, $MgSO_4$, 8 77, NaH_2PO_4 H_2O, 20 10, NaCl, 22 20, NaI, 0 0026, Na_2SiO_3 $9H_2O$, 0 61, $FeSO_4(NH_4)_2SO_4$ $6H_2O$, 1 22, $CuSO_4$ $5H_2O$, 0 44, $MnSO_4$ $4H_2O$, 0 087, $ZnCl_2$, 0 17

(figures represent percentages)

activation resulting from thymus-independent factors (lipopolysaccharides[7])

Adult male rats of a pathogen-free Sherman strain were subjected for 2 months either to a balanced normal diet (commercial chow, CNRS, Orleans) or to a protein-free diet The latter contained (per 100 g dry weight) 73 4 g sucrose, 10 g white dextrine, 10 g peanut oil, 5 7 g salt mixture (ref 8), 0 5 g choline chloride, 0 06 g inositol, and the following vitamins (in milligrams) thiamine, 1 0, riboflavin, 2 0, niacin, 20 0, Ca pantothenate, 3 0, pyridoxin, 2 0, folic acid, 0 1, vitamin B_{12}, 0 006, biotin, 0 020, tocopherol, 1 0, menadione, 1 0, vitamin A, 5,000 IU, vitamin D, 1,000 IU

Phytohaemagglutinin, (Eurobio, Paris, PHA), lipopolysaccharides from *E coli* 055 B5 (Difco, Detroit, LPS) and sheep red blood cells (SRBC) were injected into both hind foot pads of normally fed rats (3 mg of PHA, 12 5 µg of LPS, and 6 × 10^{8} SRBC injected into each foot pad) at doses double those in protein-deprived rats (respectively, 1 5 mg, 6 µg and 3× 10^{8}) The animals were killed on day 4 after injection of PHA, and on days 4 and 8 after that of LPS and SRBC All rats received intraperitoneally 1 h before death 1 µCi g⁻¹ ³H-thymidine (CEA, Saclay, specific activity 5Ci mM⁻¹)

The mean weights of a popliteal lymph node and of the spleen were determined and the incorporation of ³H-thymidine into these organs was measured in a liquid scintillation counter (Intertechnique, Paris) in the presence of scintisol The data

obtained in treated rats were compared with those of non-treated ones subjected to the same regimen On day 8, the responses were very weak even with a normal diet Therefore only the findings of day 4 were analysed

The weight of the popliteal lymph nodes and, even more, the incorporation of ³H-thymidine increased strongly on day 4 after injection of each of the three stimulants in normally fed rats (Tables 1 and 2) The increase was, however, much more marked with PHA and SRBC than with LPS The activation of the DNA synthesis was obvious even if it was not calculated per lymph node as a whole but per mg of lymphoid tissue In protein-deprived non-treated rats, the spleen showed a striking drop both in weight and in DNA synthesis, whereas in popliteal lymph nodes, the decreases in weight (Table 1) contrasted with an increased incorporation of ³H-thymidine per mg of tissue ($P < 0 05$) (Table 2) This may correspond to a selective disappearance of non-dividing, probably thymus-dependent, lymphocytes as suggested from previous data[1,9]

The lymphocytic activation induced by PHA per entire lymph node was 6 54 times weaker in protein deprived-rats than in controls (× 4 33 against × 28 31), 4 28 times weaker (× 1 24 against × 5 31) after injection of LPS, and 3 81 times weaker (× 4 29 against × 16 38) after SRBC (Table 2) Whereas the spleen was not significantly influenced by PHA, and even reduced and inactivated after injection of SRBC, on a protein-free diet, protein-deprived rats injected with LPS displayed a

Table 2 Incorporation of ³H-thymidine (c p m) by lymphocytes of popliteal lymph nodes on day 4 after subplantar injection of PHA, LPS and SRBC in normally fed rats and in protein-deprived rats

Experimental group	Popliteal lymph node c p m per entire organ Normal diet*	Protein-free diet†	c p m per mg Normal diet*	Protein-free diet†	Spleen c p m per entire organ Normal diet*	Protein-free diet†	c p m per mg Normal diet*	Protein-free diet†
Non-injected controls	1890 ±424	2,427 ±676	211 ±56	658 ±150	780,100 ±133,900	12,317 ±1,480	1,288 ±233	88 ±8
Injected with PHA	53,508 ±20,163	10,519 ±2,313	2,283 ±523	2,683 ±544	1,647,100 ±313,600	13,907 ±4,233	2,302 ±430	98 ±24
Ratio injected/ non-injected	28 31	4 33	10 83	4 08	2 11	1 13	1 79	1 11
Injected with LPS	10,042 ±1,714	3,019 ±852	786 ±104	814 ±124	1,097,200 ±351,390	70,000 ±30,535	1,527 ±480	299 ±122
Ratio injected/ non-injected	5 31	1 24	3 73	1 24	1 41	5 68	1 18	3 40
Injected with SRBC	30,960 ±3,590	10,415 ±2,958	1,641 ±267	1,563 ±365	1,083,900 ±166,700	4,739 ±2,282	1,608 ±198	48 ±37
Ratio injected/ non-injected	16 38	4 29	7 79	2 37	1 39	0 38	1 25	0 54

*Values obtained from counts made on pools of two lymph nodes
†Values obtained from counts made on pools of four lymph nodes and two spleens (two rats)

noticeable splenomegaly (1 51 times the weight of the spleen of non-injected controls, Table 1) and a very strong activation of the synthesis of splenic DNA (5 68 times the normals, Table 2) Therefore, the stimulation of the total number of lymphocytes from lymph node and spleen provoked by LPS in protein-deprived rats was not at all weaker than that observed in normally fed rats, contrary to what had been observed with the two thymus-dependent stimulants

The finding that in protein-deprived rats the lymphocytic response to LPS spread up to the spleen whereas the response to PHA remained almost completely limited to the popliteal lymph nodes, may be attributed to the higher diffusibility of the LPS molecules In the experimental rats the diffusion was probably facilitated by the atrophy of the lymph node follicles[9], which normally tend to retain the antigens[10], and thus play the role of a barrier against the dissemination of the latter The extent of the diffusion of LPS with a protein-free diet was emphasised by the appearance of necrotic areas in the liver of LPS-injected rats Normally fed rats treated with LPS and protein-deprived ones, non-injected or injected with PHA or SRBC, did not exhibit similar lesions In addition, doses of LPS double those used in the experiments described above, provoked a 44% mortality with a protein-free diet whereas corresponding doses were inoffensive to normally fed rats

The inhibitory effect of protein deprivation on the lymphocytic response to SRBC was less marked than the effect on the response to PHA Indeed, the action of PHA concerned almost exclusively the T lymphocytes whereas SRBC stimulated both the T and B cells, and the inactivation of the latter by the protein-free diet was much less pronounced than that of T lymphocytes[2]

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Increased hyaluronic acid production on stimulation of DNA synthesis in chick embryo fibroblasts

STUDIES of differentiating tissues indicate that the characteristic products of differentiated cells begin to appear only after cell multiplication has slowed or stopped[1] Therefore, it was concluded that cell multiplication and differentiated function are mutually exclusive Although this conclusion was based mainly on studies of progressively developing tissues, it has been extended to cells which have already differentiated but have retained the capacity to multiply[2] This is supported by some older studies of differentiated cells in culture[3-5] There is growing evidence, however, from studies both in vitro[6,7] and in vivo[8], that cell multiplication and differentiated function are compatible in differentiated cells capable of multiplication Here we add to that evidence by showing that the production of hyaluronic acid, a characteristic product of connective tissue, is positively correlated with the multiplication rate of chick embryo fibroblasts

Chick embryo cells were cultured as described by Rein and
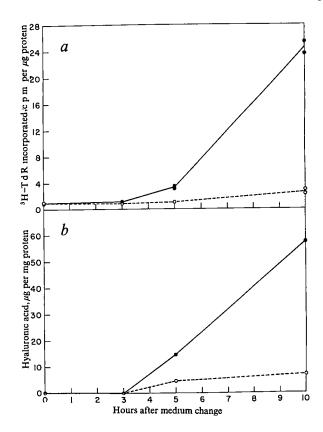
Fig 1 Four-day-old cultures in 100-mm tissue culture dishes were turned-off by changing to Medium 199, *p*H 6 8, with 2% (v/v) tryptose phosphate broth and without serum The *p*H was controlled by varying the amount of $NaHCO_3$ in the medium 16 h later the cultures were washed three times with Earle's salt solution at *p*H 6 8 Either turn-on medium (Medium 199, *p*H 7 4, with no tryptose phosphate broth and with 1% (v/v) chicken serum) or turn-off medium (Medium 199, *p*H 6 8, with no tryptose phosphate broth and with no serum) was added back to the cells *a*, The rate of DNA synthesis by turned-on and turned-off cultures was measured at various times after the medium was changed by the incorporation of ³H–TdR into acid insoluble material in a 1 h pulse DNA synthesis was measured under identical conditions by changing all cultures to Medium 199, *p*H 7 4, with no tryptose phosphate broth and no serum and with ³H-TdR (0 2 μCi ml⁻¹) for the pulse *b*, Medium was collected from subsets of the cultures at various times after the medium was changed and assayed for the amount of hyaluronic acid accumulated ●, Turned-on cultures, ○, turned-off cultures

Rubin[11] They were grown at *p*H 7 4 in Medium 199 with 2% tryptose phosphate broth and 1% chicken serum The multiplication rates of confluent cultures were controlled by manipulating *p*H and serum concentration of the medium[9,10] Low multiplication rates (turned-off cultures) were obtained by incubating cells for 16 h in serum-free medium at *p*H 6 8 Cultures were then stimulated to multiply faster (turned-on) by changing to medium (*p*H 7 4) containing serum and were compared with cultures to which fresh turn-off medium had been restored

Medium was collected from subsets of the cultures at various times after the change of medium The cells were then washed once with buffered physiological saline and the washings were added to the collected medium This mixture was lyophilised and redissolved in a small volume of distilled water NaOH was added, to a final concentration of 0 1 N, and the solution was incubated overnight at room temperature Acid mucopolysaccharides were isolated by the procedure of Bollet[13,14] as modified by Morris[15], giving 90% recovery of a known amount of hyaluronic acid added to Medium 199 Acid mucopolysaccharides were assayed by the method of Dische[16] as modified by Bitter and Ewins[17]

The relative rates of DNA synthesis were measured by the incorporation of ³H–thymidine (³H-TdR) into acid insoluble
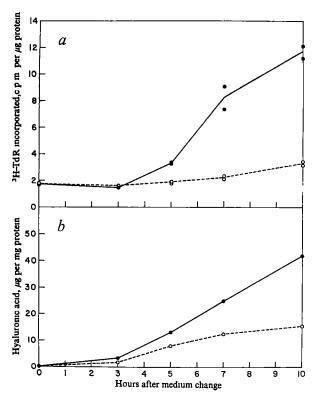
Fig. 2 Four-day-old cultures in 100-mm tissue culture dishes were turned-off by changing to Medium 199, *p*H 7 4, with 2% (v/v) tryptose phosphate broth and without serum 16 h later the cultures were washed three times with Earle's salt solution at *p*H 7 4 Either turn-on medium (Medium 199, *p*H 7 4, with no tryptose phosphate broth and with 2% (v/v) chicken serum) or turn-off medium (Medium 199, *p*H 7 4, with no tryptose phosphate broth and with no serum) was added back to the cells *a*, The rate of ³H-TdR incorporation at various times after the medium was changed, measured as in Fig 1 *b*, The amount of hyaluronic acid accumulated in the cultures at various times after the medium was changed ●, Turned-on cultures, ○, turned-off cultures

material in a 1-h pulse[11] The overall rate of DNA synthesis of a culture reflects the proportion of cells in the S phase of the cell cycle at any given time, and, therefore, is proportional to the rate of multiplication of the culture[12]

The cultures kept in medium at *p*H 6 8 with no serum continued to synthesise DNA slowly during the 10 h of measurement (Fig 1*a*) Cultures given medium at *p*H 7 4 with 1% serum had doubled their rate of DNA synthesis at 5 h, and had increased it almost tenfold at 10 h These turned-on cultures had also accumulated three times as much hyaluronic acid as the turned-off cultures at 5 h, and eight times as much at 10 h (Fig 1*b*) The insensitivity of the assay at hyaluronic acid levels less than 2 µg mg⁻¹ cell protein prevented accurate measurements of hyaluronic acid production earlier than 3 h after the medium change

To show that the differences in hyaluronic acid production were not merely an effect of *p*H, cells were turned-off by incubating them for 16 h in serum-free medium at *p*H 7 4 They were then turned-on by adding serum-containing medium at the same *p*H When serum concentration alone was varied, there was less difference in DNA synthesis rates between turned-on and turned-off cultures than when both *p*H and serum concentration were changed (Fig 2*a*) The turned-on cultures were synthesising DNA at a distinctly faster rate than the turned-off cultures at 5 h, and reached a 3 5-fold greater rate at 10 h. The amount of hyaluronic acid accumulated was detectably greater in the turned-on cultures at 5 h and had become almost three times as great at 10 h (Fig 2*b*).

These results show that the rate of cell multiplication and expression of differentiated function, as represented by hyaluronic acid production, are positively correlated with one

another We have found this situation to continue for at least 24 h after stimulation, by which time almost all the cells have undergone division Although we have not yet shown that DNA synthesis and hyaluronic acid production are positively correlated in a single cell, we conclude that there is no basis for postulating an incompatibility between multiplication and differentiated function in this sytsem

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Mitotic delay in the slime mould *Physarum polycephalum* induced by low intensity 60 and 75 Hz electromagnetic fields

INTEREST in the biological effects of non-ionising radiation has increased in the past few years, with most workers focusing on radio frequency and microwave regions of the spectrum[1-3] The ubiquity of low level 50–60 Hz electromagnetic radiation in the environment is a strong reason to investigate the biological effects of extremely low frequency radiation[4,5] We have examined the effects of low intensity 60 and 75 Hz electromagnetic fields (EMFs) and have observed delays in the mitotic cycle of the myxomycete *Physarum polycephalum* after continuous exposure Significant delays were observed after approximately 100 d of continuous exposure, corresponding to about 300 mitotic cycles

We used *Physarum polycephalum* because (1) its normal life cycle has been documented extensively both morphologically and biochemically, (2) the desired vegetative or reproductive stages can be induced at the discretion of the investigator and (3) stationary cultures undergo naturally synchronous mitosis In synchronous mitosis an entire culture of approximately 10⁹ nuclei simultaneously undergoes mitosis, permitting accurate observation of cycle timing[6,7] Synchronous mitosis occurs at predictable intervals so that the time between mitoses can be used as a sensitive indicator of EMF effects Stationary macroplasmodia can be obtained by allowing a sample of microplasmodia from submerged shake-flask cultures to coalesce on filter paper supported by milk-filter disks, growth medium is added after coalescence Both submerged shake-flask cultures and stationary macroplasmodia were exposed continuously to EMFs

Radiation fields were simulated by application of crossed electric and magnetic fields alternating in phase at 60 or 75 Hz, at levels of 0 7 V m⁻¹ and 2 0 gauss, selected to correspond to similar fields which may be generated by the US Navy's proposed Project Sanguine communications antenna[8] Our field intensities are a factor of ten greater than ground-level intensities

projected in 1970 for the Sanguine antenna, ambient 50–60 Hz fields arising from both natural and artificial sources are often within an order of magnitude of these intensities EMFs near electrical appliances in the home are often[9] in the range 10–25 gauss and 2–200 V m^{-1}

The magnetic field was generated by means of four large, thin rectangular coils placed parallel to each other at regular intervals, the entire working region was exposed to a uniform alternating field Electric fields were produced by applying a small voltage ($\sim$40 mV) to parallel stainless steel electrodes in contact with the growth medium Each container in the incubator (shake flasks and macroplasmodia dishes) was equipped with its own, individually adjustable pair of electrodes Direct electric currents were blocked by a capacitor, alternating currents through the cell were about 0 2 mA As a check on possible electrolysis of the growth medium, the pH of several flasks containing only growth medium was monitored for several days and found to be unchanged Control cultures and cultures exposed to EMFs were maintained in separate incubators at temperatures of 25 5±0 3 °C The incubators were identical in every respect except that field coils and plates were not activated in the control incubator Stock lines of *P polycephalum* were maintained as microplasmodia in submerged shake-flask cultures in both the simulation and control incubators, cultures were removed from the incubators only to transfer them to fresh growth medium or to withdraw samples for stationary macroplasmodia As soon
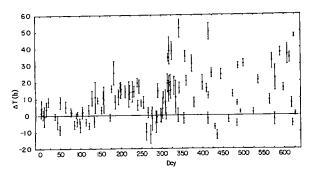
Fig 1 Relative time required for stationary cultures of *P polycephalum* to reach the metaphase configuration of mitosis compared with the number of days of continuous exposure to 75 Hz EMFs (0 7 V m^{-1}, 2 0 gauss) Each point represents the average of 10 cultures from which the average of 10 control cultures for that day has been subtracted The error bars are 95 % confidence limits

as stationary cultures were made, both submerged and stationary cultures were returned to simulation and control incubators To eliminate small day-to-day variations in mitotic cycle timing an identical number of stationary control cultures was set up each time observations were to be made on a group of experimental cultures All cultures were handled in an identical fashion using aseptic techniques The experimental parameter was the time required for stationary cultures to reach metaphase of the second mitotic division measured from the addition of growth medium The second division was used because it occurs approximately 14 h after feeding, so that observations can be scheduled in the morning Periodic measurements of the interval between the second and third mitoses confirmed cycle delays ascertained from observations of the second mitosis alone

After a culture had been in a 60 Hz EMF for 80–100 d a significant mitotic delay became evident, while the same effect took 100–120 d in a 75 Hz EMF To determine whether the change in the mitotic cycle was reversible, several cultures were moved from the simulator to the control incubator The delay persisted for 30–60 d during which time the mitotic interval slowly converged to the control interval No significant delay was observed in cultures exposed for 200 d to weaker 75 Hz EMFs of 0 15 V m^{-1} and 0 4 gauss Furthermore, when cultures with a delayed cycle were put in these fields after being taken

from the higher intensity 75 Hz simulator, the delay slowly decreased and became insignificant after 40 d

Figure 1 presents data for cultures exposed to 75 Hz EMFs, data for 60 Hz fields are similar The dip in the curve during days 260 to 310 results from difficulties with control cultures, which became excessively slimey and underwent mitosis asynchronously, on day 310 they were replaced with alternative cultures that had been maintained in the control incubator from day 1 as a reserve

The data were analysed by two statistical tests, both test the null hypothesis that experimental and control populations are identical An analysis of variance was used to apply a test of significance simultaneously to differences between all possible pairs of day-averages In other words, the question posed is can a horizontal line be drawn through the data (not necessarily at $\Delta = 0$) ? A signed ranks test was also used; in the test, the error bars on the data points are ignored and the number and extent of positive differences are compared with negative differences to determine if any net effect is present On some days mitosis took less time to occur in experimental than in control cultures This test asks whether the reversal happened often enough to accept the null hypothesis Our analysis shows that for both 60 and 75 Hz data, both statistical tests indicate that the null hypothesis can be rejected with at least 95 % confidence The 75 Hz data for days 260–310 were included in our analyses

We conclude that low level, low frequency EMFs can influence biological systems This conclusion agrees with findings from other laboratories[4,5] Two important features of our observations deserve emphasis First, delay in the mitotic cycle does not show up until after the cultures have been exposed for long periods Second, the delay does not disappear immediately when the cultures are removed from the field simulator

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Rapid quantitation of membrane antigens

STUDIES of cell-surface antigens are important in several fields of biological research, notably transplantation and tumour research The most commonly used tests for such antigens involve assays requiring complements, and the subsequent measurement of cellular damage by the release of radioactive isotopes or the uptake of supravital dyes Radioimmunoassays which measure directly the antibody–antigen reaction in such studies would have considerable advantages Although it is possible to label antibody molecules directly, such attempts have so far been unsuccessful because of high background binding values Furthermore, variations in the composition of the antisera would make each antiserum a unique target for radiolabelling with consequent variations in results We have

Table 1 Use of ^{125}I-protein A for rat, mouse and human cell typing

Rat target	Sera	c p m.	Mouse target	Sera	c p m	Human target*	Sera†	c p m
Lewis	DA→Le	7,400	H−2 b	k→b	4,676	2, 5, 12, 19	1	282
(Le)	Le→DA	340				2, 5, 12, 19	7	183
						2, 5, 12, 19	2	1,538
DA	DA→Le	541	H−2 k	k→b	604	2, 5, 12, 19	12	1,045
	Le→DA	6,920				2, 5, 12, 19	28	761
						3, 7, 28, W15	1	265
DA×Le	DA→Le	3,810	k×b	k→b	2 514	3, 7, 28, W15	7	1,073
(Fi)	Le→DA	3,520	(Fi)			3, 7, 28, W15	2	791
						3, 7, 28, W15	12	299
BD	DA→Le	542	P815	k→d	7,771	3, 7, 28, W15	28	1,140
	Le→DA	3,376	H−2 d			1, 3, 7, W15	1	934
	NRS	387	NMS		470	1, 3, 7, W15	7	881
						1, 3, 7, W15	2	300
						1, 3, 7, W15	12	317
						1, 3, 7, W15	28	114

Cells in tubes or microtitre test plates were incubated with (25 μl) antisera for 30 min at 4 °C, washed and incubated with ^{125}I-labelled (50,000 c p m) protein A or ^{125}I-labelled whole bacteria After a further 30 min at 4° C the cells were washed and counted Cells on plates were washed by centrifugation followed by shaking out the supernatant, and transferred to tubes for counting Rat, mouse and human sera were used at dilutions of 1 125, 1 100 and 1 5, respectively The P815 mouse mastocytoma cells were from *in vitro* cultures
*HL-A type was determined by Dr Bertil Lindblom at the University Hospital using "Uppsala" antisera and a trypan blue cytotoxicity test
†Anti-HL-A sera from McIndoe Memorial Research Unit, East Grinstead, Sussex, UK
Arrows represent anti

tried to circumvent this using a radiolabelled homogeneous marker for IgG molecules produced by certain bacteria

Protein A from *Staphylococcus aureus* Cowan I binds specifically and with high affinity to the Fc portion of most mammalian IgG subclasses[1] In our assay ^{125}I-labelled *Staphylococcus aureus* Cowan I or labelled protein A were incubated with cells precoated with a wide range of antisera to membrane components Radioactivity (c p m) provides a measure of the number of IgG molecules bound per cell and hence the number of specific probe–surface markers The value of protein A as an immunological probe lies in its versatility, as in our example, in which a single ^{125}I-labelled protein A preparation has been used to study antigens on human, rat and mouse cells precoated with rabbit and monkey as well as with alloantisera

The preparation and iodination of protein A have been described elsewhere[2] Whole bacteria were isolated in a similar manner but were simply washed free of excess iodine We have used this ^{125}I-protein A to develop a complement-free radioimmunoassay applicable to a wide range of mammalian membrane antigens Table 1 shows how the method can be used to type human, mouse and rat lymphoid cells with alloantisera or xenoantisera The data for rat and mouse show the expected gene dose effect as observed under optimal reaction conditions using both antibody and ^{125}I-protein A in excess Such a gene dose 'control' provides a good test of the function of any individual experiment Cross reaction of Lewis anti-DA with BD rats is also clearly shown

Thus, for rat and mouse systems, using inbred material, the
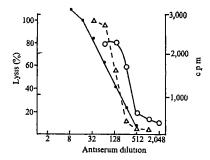
Fig. 1 Triplicate dilutions of rabbit anti β-2 microglobulin were assayed for ability to bind to 10^5 human T cells by three separate methods The ^{51}Cr assay[4] (○) and trypan blue test (△) are plotted as percentage lysis because these assays measure cell death The protein A assay (●) measures directly the number of antibody molecules bound per cell, and the cells remain viable throughout, c p m bound at each antiserum dilution are therefore plotted Sensitivity of the IpA assay can be varied simply by changing the specific activity of the iodinated reagent
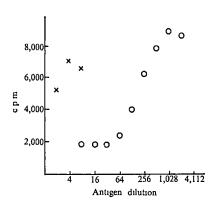
Fig. 2 HL-A antigen isolated by standard procedures[5] from splenic lymphocytes after HN$_4$Cl removal of red cells, was diluted with saline and a constant amount of antibody added (sufficient to give 10,000 c p m per 10^6 cells) with ^{125}I-protein A After 2 h at 4° C, 10^5 cells were added per well of the test plate and incubation continued for a further 30 min at 4° C Plates were centrifuged, washed and incubated with protein A in the usual way ○, HL-A 2 antigen, ×, HL-A 12 antigen Antiserum used, A macacus anti HL-A 2 serum[5]

results of this assay were highly satisfactory Table 1 shows that the assay can also function in the HL-A system, although sizeable binding occurs between supposed 'negative' serum-cell combinations Extensive cross reactions can occur, however, between HL-A antigens, this is in agreement with the cross reaction found[3] between HL-A2 and HL-A28 When we compare (Fig 1) the protein A assay with conventional assays for HL-A such as trypan blue or ^{51}Cr using microassays in plates[4], we found a positive correlation between the three tests The ^{125}I-protein A assay has a similar sensitivity to that of the trypan blue or ^{51}Cr assays, but is more linear and thus more precise than the other two tests

The ^{125}I protein A assay was also tested for its suitability in testing for soluble HL-A determinants in inhibition assays Figure 2 indicates that the protein A assay is very suitable for studies of HL-A antigens in soluble form The inhibitor assay obtained was again more linear than inhibition of trypan blue or ^{51}Cr lysis

Whole *Staphylococcus aureus* bacteria bind to the Fc region of IgG and there seemed to be no reason why bacteria could not be heavily labelled, for example, for cell suicide experiments Also cells carrying whole bacteria can be readily separated by Ficol gradient centrifugation (V Ghetie, K Nilsson, and J Sjoquist, unpublished), from those not carrying bacteria In preliminary studies we have iodinated bacteria and used them in an identical manner to that used for protein A Figure 3
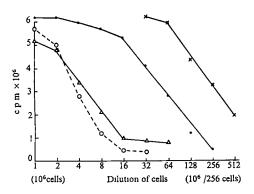
Fig 3 Dilutions of T and B cells in microtitre test plates were assayed directly for binding of ^{125}I-labelled whole bacteria or preincubated with rabbit anti-Ig sera and assayed for binding of ^{125}I-labelled whole bacteria The assay conditions are as described in the footnote to Table 1 ○, T cells+autologous serum, △, T cells incubated with anti-Ig, ●, B cells + autologous serum, ×, B cells incubated with anti-Ig

shows such an assay involving human B and T cells B cells or T cells separated by T cell rosettes[6] were incubated with normal human sera or rabbit anti-Ig sera before testing

Using ^{125}I-labelled *Staphylococcus aureus* Cowan 1 strain bacteria, human B lymphocytes incubated with anti-Ig antibodies bind approximately four times the number of bacteria than do uncoated B lymphocytes, and some 100 times more than T lymphocytes treated or not with anti-Ig Note that our antisera contained antibodies against light chains (both χ and λ chains) but still failed to react with human T lymphocytes as assessed by this assay (in other words, there is no difference between T lymphocytes treated or not with anti-Ig)

We have described mainly how ^{125}I-protein A assays can be used to supplement conventional methods in mammalian systems for assaying cell-bound and soluble antigens Several points make protein A assays unique, perhaps the most important being the specificity of binding to Ig molecules Protein A will only bind to IgG 1, 2 and 4 in human cells, there being no binding to IgG 3 or IgM for example In general IgG 1, 2 and 4 account for about 92% of human IgG We have carried out a series of experiments in which human cells precoated with alloantibodies are then coated with rabbit anti-IgG before addition of protein A Such sandwich techniques, however, hardly increase specific binding in the human system In the rat and mouse system it is not known exactly what percentage of complexed IgG binds protein A but our sandwich assays indicate it to be 60–100% in both species Calculation of the absolute number of antigen molecules per cell are, of course, affected by such considerations but this is easily checked by including a sandwich assay as described above The assays described in Table 1 and Figs 1 and 2 approach linearity and we expect total linearity when the assays improve technically This makes them much more amenable to kinetic studies and also to studies of cross reaction than complement-dependent assays In addition cells are live at the end of the experiment and loss of counts from cells can be easily monitored

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Membrane changes in a circadian system

Njus *et al*[1] have recently suggested a membrane model of a biological clock and propose that the clock mechanism is a feedback oscillator in which the ion transport channels envisaged in the fluid mosaic model of cellular membranes[2] are involved directly It is postulated that the proteins which make up these channels respond to changes in concentration gradients of specific ions in the membrane, by grouping to form transport channels when the gradient is small, and dispersing to non-transporting modes when the gradient is large Thus the protein distribution in the membrane could be regulated by the oscillating ionic gradient and could itself regulate the ionic gradient to complete the feedback pathway and drive the oscillation It has also been suggested[3-5] that oscillatory changes at cellular membranes may be directly associated with the clock mechanism, based on observations that the phase or the period of the oscillation can be changed by treatments which affect the passage of ions through membranes

We provide direct experimental evidence for cyclic changes in membrane properties in a circadian system from studies of the pulvinule cells at the base of the leaflets of clover, *Trifolium repens* L Swelling of the cells in the adaxial portion of the pulvinule and compression of those in the abaxial portion cause the leaflet to open, and a reversal of this process causes it to close We have shown[6] that the volume changes and the resulting leaf movements are caused by an endogenous circadian oscillator located in the pulvinule Further observations (which will be reported more fully elsewhere), have shown that redistribution of cations (principally K^+) within the pulvinule accompanies these variations in volume

Table 1 Ion concentrations in pulvinal tissue (meq per kg wet weight)

		Adaxial	Abaxial	Adaxial−Abaxial
K	Day	*193 7±11 7*	167 8± 8 3	25 9± 9 2
	Night	140 3±12 5	*197 4±31 2*	−57 1±20 8
Na	Day	*19 5± 2 7*	18 5± 2 5	0 9± 2 2
	Night	19 5± 2 4	*24 6± 5 7*	−5 2± 4 1

Mean ion concentrations (±s e m) of abaxial and adaxial segments of pulvinal tissue of clover in LD 12 12 The swollen (convex) segment is shown in italics

Table 1 gives the ion concentrations in pulvinal tissue for plants grown in a 12 h light, 12 h dark cycle (LD 12 12) at 20 °C The pulvinules were sectioned into abaxial and adaxial segments at about the middle of the light and the dark intervals, and analysed using flame photometry The cation component is predominantly potassium and examination of sections stained with sodium cobaltinitrite show that most of it is in the cortical pulvinal cells which occupy at least 80% of the volume of the segments As the potassium concentration is significantly greater on the swollen side where there are fewer cells per unit volume, it is clear that each enlarged cell contains very much more potassium than each cell on the contracted side Thus there must be large tidal movements of potassium across the pulvinal membranes during the daily cycle Using a different experimental technique, Satter, Galston and co-workers conclude that similar potassium changes occur in *Albizzia*[7] and *Samanea*[8]

Radioisotope tracer studies reveal that considerable changes in potassium transport occur in pulvinal tissue during the cycle Leaves were soaked in a medium containing ^{42}K and ^{45}Ca for 2 h and washed for 30 min, their pulvinules were sectioned

into abaxial and adaxial segments The activity of the segments from groups of several pulvinules was measured immediately, and again after the short lived ^{42}K had decayed, to determine separately the influxes of the two ions The double labelling experiment was performed because it was found that calcium influx is approximately proportional to the dry mass of the tissue segments, which is more appropriate for comparison purposes than the wet mass which fluctuates as the cells expand and contract Since the dry mass is difficult to measure accurately for a small number of tissue segments, the potassium influx could more conveniently be expressed as its ratio to the calcium influx

Figure 1 shows the variation in influx ratio for the two sides of the pulvinule during an LD 12 12 cycle The influx of potassium is relatively much greater when the cells are expanded than when they are contracted Such differences are expected for cells in the process of swelling or shrinking The influx ratio is, however, significantly greater or less than unity for much longer periods which commence before the leaf is observed to move and continue for several hours after the leaf is fully opened or fully closed It has not been practicable to obtain corresponding efflux data
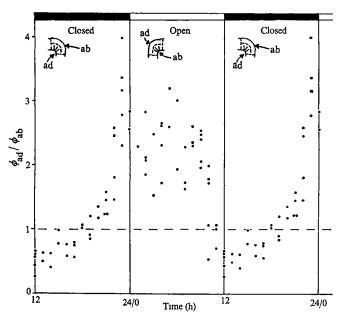
Fig 1 Variation of pulvinal influx ratio with time (h after onset of light) for clover grown in LD 12 12 at 20 °C φ_{ad} is the K$^+$ influx per unit influx of Ca^{2+} in adaxial segments of pulvinules φ_{ab} is the corresponding quantity for abaxial segments Each point is the influx ratio for groups five to ten pulvinules One and a half cycles of the oscillation are shown

These data show that the transport properties of the membrane are changing in a cyclic manner in LD 12 12 The changes may be in membrane permeability to specific ions or in an active transport system The phase of the influx cycle is about 3 h in advance of both the leaf oscillation and the LD cycle This phase relationship is significant since it strongly suggests that the change in membrane properties is a preliminary step in the sequence of changes responsible for leaf movement, presumably by osmotically varying the volume of pulvinal cells through changes in their potassium content

The phase relationship in LD 12 12 also suggests that the flux changes are not a direct response to the light cycle This is confirmed by transferring plants to conditions of constant light and temperature (LL), following which we found that the influx oscillation continues for at least two cycles The period of these oscillations is about 27 h, which is the same as the period of leaf oscillations under these conditions[6] We therefore conclude that the oscillatory system controlling both potassium influx and leaf movement is circadian and under endogenous control

Attempts have been made to measure the transmembrane

potentials of pulvinal cells The internal potential of cells on the abaxial side is $-71\,6\pm2\,0$ mV early in the contracted phase and $-102\,1\pm6\,4$ mV early in the swollen phase (P S Targett, unpublished) Cells on the adaxial side show corresponding changes but opposite in phase The time course of these potential changes has not yet been established in detail, but it corresponds qualitatively to what is expected if the membrane potential is determined principally by the passive diffusion of potassium, since the magnitude of the potential is larger when the internal potassium concentration, and presumably the concentration gradient, is higher

In their circadian oscillator model, Njus *et al*[1] do not suggest a mechanism for the dispersal of the proteins forming the transport channels when the concentration gradients are large We propose that the electric field in the membrane may act as the dispersing agent by supplying the necessary forces The average strength of the transverse field is large ($\simeq 10^7$ V m^{-1}), but the field lines are likely to be distorted in the vicinity of transport channels where hydrophilic pathways will have much lower resistance per unit area than the bulk of the membrane The distorted field will have components in the plane of the membrane The proteins immersed in the membrane, which make up the transport channels, carry charged endings and therefore would experience forces resulting from a lateral field which could cause them to disperse or undergo changes in configuration when the electric field increases

It is well known that depolarisation of the excitable membranes in nerve[9] and the large Characean cells[10] causes large changes in the passive fluxes of specific ions, resulting in an action potential Hyperpolarised membranes also show marked changes in their conductance to specific ions[11] Eskin[12] has suggested that the biological clock in *Aplysia* may involve neuronal depolarisation

Experimental support for a circadian oscillator located at the cellular membrane in some biological organisms does not preclude the existence of a different system sited elsewhere (for example, in the nucleus[13]) which controls other oscillatory characteristics in these or other organisms If rhythmicity gives a biological system an evolutionary advantage, it is quite possible that different mechanisms have arisen independently

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Effects of L-dopa metabolites at a dopamine receptor suggest a basis for 'on-off' effect in Parkinson's disease

ALTHOUGH L-dopa is the most effective therapeutic agent available for the control of the akinesia of Parkinson's disease, long term therapy may be associated with a variety of therapeutic problems[1] One problem seen in some patients is a progressive loss of therapeutic response which may be associated with abnormal involuntary movements[1] A state may develop in which a good response to the drug alternates with periods of akinesia and rigidity[1-4] This has been called the
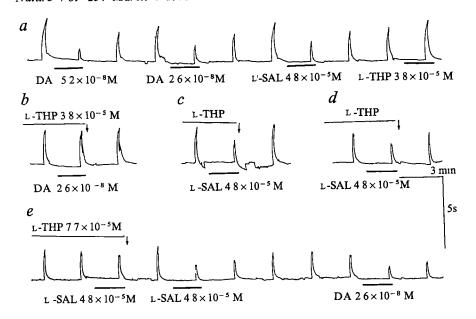
Fig. 1 Effects of dopamine (DA hydrochloride, Mann) L-salsolinol (L-SAL) and L-THP on intestinal responses evoked by electrical stimulation of gut of *Tapes watlingi* Guts were prepared as before[9], incubated in artificial seawater and stimulated electrically by means of transmural electrodes Unlabelled upward deflections represent contractions of the gut resulting from a 5-s train of square wave pulses (1 ms, 20 Hz, 10 V) The black bars indicate the periods for which DA, L-SAL or L-THP were present in the 2-ml organ bath *a*, Perfusion with artificial seawater alone, *b*, *c* and *d*, Responses 4 min after L-THP ($3\ 85 \times 10^{-5}$ M) had been added to the bath *e*, L-THP ($7\ 7 \times 10^{-5}$ M) was also present After the times marked by arrows L-THP was washed from the bath

'on-off' effect or akinesia paradoxica[1] While low blood levels of L-dopa may explain the onset of akinesia ('off' effect), this cannot explain the sudden reversal of patients' akinesia ('on' effect) which may occur without additional dosage of L-dopa[2,3]

Several mechanisms have been proposed to explain both the progressive loss of efficacy and the 'on-off effect, but so far there is little supporting data[1-4] One suggestion has been that complex metabolites of L-dopa, which are structurally similar to apomorphine, may antagonise the effects of dopamine[6] Two complex metabolites of L-dopa, tetrahydropapaveroline (THP) and salsolinol (SAL) have been identified in the urine of patients with Parkinson's disease who had been treated with L-dopa[6] THP has also been identified in rat brain after administration of L-dopa[7] It has been suggested, however, that THP, SAL or similar tetrahydroisoquinoline compounds may behave as dopamine (DA) agonists and contribute to the therapeutic effect of L-dopa[6,8]

We have now found that THP may antagonise the action of DA and SAL at a DA receptor We have investigated the activity of DA and the isomers of THP (hydrochloride salt), SAL (hydrobromide salt)[13] and amphetamine at DA receptors in the isolated gut of the mollusc *Tapes watlingi*, which contains both dopaminergic neurones and specific dopamine receptors[9] Figure 1 shows the responses to electrical stimulation in one gut preparation in the presence of DA, L-SAL and L-THP Contraction was inhibited by DA ($2\ 63 \times 10^{-8}$–$5\ 26 \times 10^{-8}$ M) and L-SAL ($4\ 8 \times 10^{-5}$ M), but was hardly affected by L-THP ($3\ 85 \times 10^{-5}$–$7\ 7 \times 10^{-5}$ M) (Fig 1*a*) The DA-like effect of L-SAL suggested that this compound was a weak agonist at the DA receptor In two out of six preparations in which L-THP had no DA-like effect, the amplitude of contraction increased It is interesting that DL-THP has been reported to have no DA-like effect on adenyl cyclase from rat striatum[10]

In the presence of L-THP ($3\ 85 \times 10^{-5}$ M), the inhibitory response to DA ($2\ 63 \times 10^{-8}$ M) was abolished (Fig 1*b*), while the response to L-SAL ($4\ 8 \times 10^{-5}$ M) was unaffected (Fig 1*c* and *d*) A larger concentration of L-THP ($7\ 7 \times 10^{-5}$ M) did reduce the response to L-SAL (Fig 1*e*) In two further preparations, L-THP ($2\ 31 \times 10^{-5}$ M and $7\ 7 \times 10^{-5}$ M) completely antagonised the inhibitory effects of L-SAL ($2\ 4 \times 10^{-5}$ M and $9\ 6 \times 10^{-5}$ M, respectively) These concentrations of L-THP also antagonised DA at an equipotent concentration ($5\ 26 \times 10^{-8}$ M) Clearly, the effects of both DA and L-SAL can be antagonised by L-THP

D-THP was relatively inactive as a DA antagonist in concentrations ten times greater than those at which L-THP was effective It was equally effective when the concentration of D-isomer was 30 times greater than that of the L-isomer Both D and L-THP have antagonist activity at DA sensitive receptors

associated with adenyl cyclase of the caudate nucleus[11] When tested on five gut preparations, DA was 10^3 times more potent than equimolar concentrations of L-SAL In two experiments, the optical isomers of SAL were approximately equipotent as DA agonists The optical isomers of amphetamine (amphetamine sulphate, Sigma) had a DA-like effect on the preparation Again, the isomers were equipotent and exhibited molar potencies similar to those measured for isomers of SAL Recent studies of the DA-sensitive adenyl cyclase system in the rat striatum revealed either no DA-like activity of SAL[10] or DA antagonism by L-SAL[11] The reasons for the failure to demonstrate DA agonist activity with D- and L-SAL in DA-sensitive adenyl cyclase of the rat striatum is not clear. One possible explanation is the limited range of concentrations tested Alternatively, if SAL acts as an indirect agonist, the lack of dopaminergic nerve terminals in the preparation would exclude such an effect

Histochemical fluorescence[9] has indicated that the dopaminergic system in the gut of *Tapes watlingi* consists of varicose nerves with a presynaptic capacity for the synthesis, release and uptake of DA There are, in addition, postsynaptic receptor sites which respond to DA agonists As SAL lowers the accumulation and retention of tritiated DA in rat brain synaptosomes[12], it is possible that the optical isomers of SAL mimic the effect of DA on the gut of *Tapes* by augmenting its release and/or inhibiting its reuptake

Thus we have demonstrated the diverse pharmacological activity of known metabolites of L-dopa D- and L-SAL are dopamine agonists and L-THP is a potent dopamine antagonist

We suggest that the therapeutic effects of L-dopa that are presumed to be mediated by DA may be modulated by metabolites of DA itself Thus therapeutic success or failure with L-dopa in Parkinson's disease may depend in part on the relative rates of production and accumulation of these or similar metabolites in the brain

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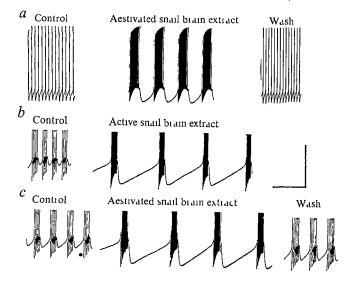
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Peptide factor extracted from molluscan ganglia that modulates bursting pacemaker activity

NEUROHORMONES have been suggested as long term regulators of the physiology and biochemistry of nervous tissue[1-3] Long term changes in the electrophysiology and biochemistry of a specific neurosecretory cell in the land snail *Otala lactea*[4,5] suggested such a form of long term hormonal regulation, and in fact several vertebrate neurohypophysial peptides cause long term changes in the membrane properties of this cell[6] The regulatory effects observed involve the induction or potentiation of bursting pacemaker potential (BPP) activity, resulting in net excitation of the cell These effects are different from the conventional transient conductance changes produced by various putative transmitters on these cells We have now found a naturally occurring substance present in the snail brain that produces similar long term changes in the membrane properties of this neurosecretory cell The results provide evidence for a peptide neurohormone which may function physiologically in the seasonal regulation of the physiology and biochemistry of a neurosecretory cell

Crude extracts of molluscan nervous tissue were prepared by removing the circumoesophageal ganglia from *Otala lactea* and *Aplysia californica*, and rinsing the tissue in physiological saline For comparison, the buccal retractor muscle and foot muscle of the snail were also excised The tissue was blotted on filter paper, weighed, and transferred to a 5% acetic acid solution in a glass homogenising tube, immersed in a bath of boiling water

Fig 1 Effects of snail brain extracts on BPP activity of cell 11 *a*, Membrane potential traces of cell 11 (taken from a dormant snail) exposed to an extract of whole brain from aestivated snails Under control conditions the snail could not generate BPP activity A 5-min bath application of the extract induced BPP activity Washing for more than 1 h with extract-free saline restored the activity to approximately its control levels *b* and *c*, Extracts of brains from either aestivated or active snails augmented the BPP activity already present in cell 11 taken from an active snail Prolonged washing restored the membrane potential behaviour to its control state Calibration 40 mV, 12 s

a Control   Aestivated snail brain extract   Wash

b Control   Active snail brain extract

c Control   Aestivated snail brain extract   Wash

The tissue was heated for 10 min in this solution, and then homogenised twice using a Teflon-tipped pestle The ratio of extraction solvent to tissue was 20 1 (v/v, tissue specific gravity assumed to be 1 0) The homogenate was centrifuged at 12,000g for 15 min, and the supernatant was removed and lyophilised The dried material was resuspended in either snail physiological saline[4] (80mM NaCl, 4mM KCl, 10mM $CaCl_2$, 5mM $MgCl_2$, 10mM Tris, pH 7 8) for assay, or in 5% acetic acid for separation on Sephadex G-10 (0 9×43 cm) or G-25 (0 9×30 cm) columns The resulting fractions were lyophilised and dissolved in snail saline for assay Sephadex columns were standardised with blue Dextran 2000, yellow Dextran 20 (Pharmacia), cytochrome *c*, vitamin B_{12}, colbalt chloride, lysine-vasopressin, oxytocin and various amino acids as markers Elution volumes of the peptides and amino acids were determined by spotting aliquots of the various fractions on Whatman 3 MM filter paper, and spraying with ninhydrin

Assays were performed on the peptide-sensitive neurosecretory cell (cell 11)[6], taken from either active or dormant snails Dissection of the ganglion, identification of cell 11, and recording techniques were as described previously[4-6] Cells taken from dormant snails have different membrane properties from those taken from active snails[5], and therefore the samples to be tested were applied to both types of preparations The basal level of spontaneous electrical activity in the cell was usually recorded for 30–60 min before application of the material to be tested The 1-ml samples (and control samples) were added by pipette to the 4 0 ml saline bathing the snail ganglion After observation of the effect, the preparation was washed with 20 volumes of physiological saline

After application of the extract to the medium surrounding a cell from a dormant snail generating a beating pacemaker rhythm, BPP activity was induced (Fig 1*a*) relatively rapidly (seconds to minutes) Application of the extract to a cell from an active snail potentiated BPP activity already present (Fig 1*b* and *c*) The response of the cell was initially increased during the washing Prolonged washing of the cell (> 1 h) in saline was usually required to reverse the effects of the extract Thus, the factor(s) in the extract had a long term effect on the BPP activity of cell 11 similar to that observed with neurohypophysial peptides[6] The increase in BPP activity observed immediately on washing may have resulted from the removal of inhibitory substances (for example, the putative transmitters dopamine and glutamate, both of which inhibit BPP activity in cell 11) which acted only while the extract was in the bath Extracts with activity were obtained from ganglia of active (Fig 1*b*) and dormant (Fig 1*c*) snails and from the circumoesophageal ganglia of *Aplysia californica* (not illustrated) Similar extracts of the buccal retractor and foot muscles of the snail had no effect, suggesting a brain-specific substance

Preliminary attempts were made to characterise this factor(s) The BPP activating factor was lost during dialysis, but maintained full activity when exposed to 100 °C for 10 min or to 50% acetic acid These treatments had similar effects on vasopressin's ability to induce or enhance BPP activity Fractionation of the crude extract on Sephadex G-10 showed that the active factor(s) was eluted in the exclusion volume (V_E) (Fig 2*a*) whereas a potent inhibitory factor was eluted in subsequent elution volume (V_1) Since Sephadex G-10 excluded molecules with molecular weights greater than 700, we conclude that the BPP inducing factor is greater than 700 while most of the inhibitory material is smaller In standard runs on Sephadex G-10, lysine-vasopressin and amino acids eluted in V_E and V_1, respectively Fractionation of the crude extracts on Sephadex G-25 (which excludes molecules greater than 5,000) yielded an inactive exclusion volume (V_E) (Fig 2*b*) BPP-inducing activity was in the V_3 fraction (Fig 2*b*) (This fraction also contained the vasopressin and oxytocin in standard column runs) In addition, there seemed to be a larger inhibitory factor in V_2 as well as inhibitory material in V_3 (since washing the cell free of V_3 further enhanced BPP) The latter inhibition most likely corresponds to the components found in V_1 from Sephadex G-10

Fig. 2 Sephadex chromatographic separation of snail brain extracts *a*, Application (arrow) of fraction V_E (exclusion volume) from Sephadex G-10 induced BPP activity (with large amplitude slow oscillations, which generated high frequency spike activity), whereas application of the V_1 (included volume) caused inhibition of spontaneous activity and hyperpolarisation of cell 11, which was reversible washing with fresh saline (upward arrow) *b*, Application of various frations from a Sephadex G-25 separation showed that the active factor(s) was in the V_3 fraction Another, higher molecular weight factor with a slight inhibitory effect on the BPP was eluted in V_2 Note that washing the cell after application of V_3 produced an enhancement of the BPP (this may have been caused by the presence of amino acids which eluted in both V_3 and V_4 —see text for further details) Calibration 40 mV, 12 s (2 min during part of control, and V_1 wash in (*a*) and V_2 wash in (*b*)

Fig 3 Effects of proteases on the BPP-inducing factor *a*, Electrical activity in cell 11 before (control) and after application of trypsin-treated factor *b*, Same as (*a*), except the material was treated with chymotrypsin *c*, Electrical activity in cell 11 before (control), after application of the Pronase-treated extract, and after application of an equivalent amount of the extract not treated with Pronase (untreated) Calibration 40 mV, 12 s

(Fig 2*a*), since in the standard runs amino acids eluted in the V_3 fraction The final fraction (V_4) was inactive

The pharmacological effects and gel filtration behaviour of the activating factor in the extract suggest that the extracted factor might be a peptide We therefore investigated whether the extracted factor could be inactivated by proteases Aliquots of the active V_E fraction from Sephadex G-10 (Fig 2*a*) were incubated with immobilised trypsin or chymotrypsin (Worthington) or Pronase (Calbiochem) Preliminary experiments showed that all these proteases inactivated the BPP-inducing activity of vasopressin (Pronase and chymotrypsin inactivated oxytocin, but trypsin did not), and that enzyme treatment itself did not affect the assay Incubation of the active extracts with either chymotrypsin or trypsin did not inactivate the BPP-inducing factor (Fig 3*a* and *b*), but Pronase treatment abolished activity (Fig 3*c*) These results indicate that the active material was a peptide, but not vasopressin or oxytocin since both of these peptides were inactivated by chymotrypsin (and trypsin, in the case of vasopressin)

In summary, we have isolated from molluscan ganglia a factor(s) which can either induce or enhance BPP activity in a specific neurosecretory cell in *Otala lactea* The activating effects of this peptide factor are similar to the actions of certain vertebrate neurohypophysial peptides in that both are elaborated by nervous tissue, cause long term changes in the membrane properties of a specific cell in the snail, the result of which is BPP activity, are water-soluble, are stable to heat and acid, are dialysable, and co-elute from Sephadex G-10 and G-25 The extracted factor differs from the neurohypophysial peptides in its resistance to inactivation by trypsin and chymotrypsin These results suggest that peptides similar, although not identical, to the neurohypophysial peptides function naturally in the modulation of the physiological properties of nerve cells in the snail

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Temperature adaptation in myosin of Antarctic fish

A RELATIONSHIP exists between the thermostability of fish skeletal muscle myosin, actomyosin and myofibril preparations and the environmental temperature at which the fish lives[1-3] Compared with those isolated from mammals and warm sea fishes, the myosins isolated from cold water species readily aggregate on storage, are more sensitive to denaturation by heat and urea, and quickly lose all ATPase activity following preparation[4,5] We have compared the properties of myofibrils and myosin prepared from the white muscle of an Antarctic fish, *Notothenia rossii*, South Georgia, British Antarctica, with homologous preparations from a tropical species, *Amphiprion sebea* (Indian Ocean, 23–27 °C) We suggest that the thermal lability of cold-adapted fish myosins arises from differences in the higher order in the structure of the molecule, this is probably an evolutionary response to attain high catalytic efficiency at low temperatures

The myofibrillar ATPase enzyme from the Antarctic fish (Fig 1) has a much higher specific activity at low temperatures (0–10 °C) than does the tropical fish The apparent energies of activation for the reactions between 0 and 18 °C were
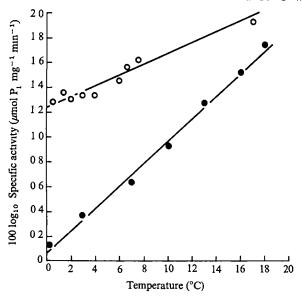
Fig. 1 The effect of temperature on the specific activity of white muscle myofibrils of *Notothenia* (O) and *Amphiprion* (●) Myofibril preparations were used for ATPase activity studies because the ATPase of myosin isolated from cold water fish is extremely unstable Myofibrils were prepared from fish expaxial white muscle[6], excluding superficial red muscle which has a different myofibrillar ATPase activity[7] Studies on the thermal denaturation of myofibrillar ATPase activity were carried out between 25 and 37 °C by incubating myofibrils (1mg ml⁻¹) in 0 05 M KCl, 40 mM Tris-HCl (*p*H 7 5) Myofibrils were added to an 18-fold excess of continuously stirred medium and an initial sample taken 0 5 min later Subsequent samples were taken at appropriate intervals and pipetted into tubes at 0 °C to prevent further inactivation Myofibrils partially inactivated by exposure to high temperatures were assayed for ATPase activity at 18 °C The assay for ATPase activity was performed in 1 5 ml 40 mM Tris-HCl (*p*H 7 5) with 6 mM ATP, 6 mM MgSO₄ and 0 2 mM CaCl₂ at *I* = 0 124 (adjusted with KCl), and at a myofibril concentration of 0 4–0 5 mg ml⁻¹ The reaction was terminated with 1 5 ml TCA and the liberated phosphate was determined[8] Determinations of myofibrillar ATPase activity were made at temperatures between 0 and 18 °C in the same assay conditions Appropriate controls and reagent blanks were included in all experiments

lower in the case of the Antarctic fish, 7 4 kcalorie mol⁻¹ compared with 17 3 kcalorie mol⁻¹ *Notothenia* myofibrils lost 95 % of their activity after 7 min incubation at 37 °C, whereas those of *Amphiprion* still retained 45 % of their original activity after 20 min incubation The denaturation reaction (Fig 2) followed first order reaction kinetics only in the case of the Antarctic fish In the tropical fish there was an initial activation of the ATPase activity lasting approximately 12 min, the activation in this species occurred between 37–32 °C A similar activation effect of much shorter duration was noticed in *Notothenia* at somewhat lower temperatures (32–27 °C)
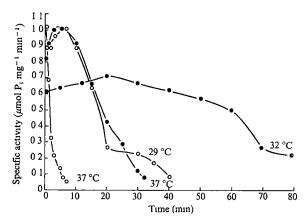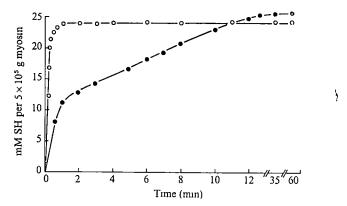
Fig 2 The thermal inactivation of myofibrillar ATPase of *Notothenia* (O) at 37 °C and 32 °C and of *Amphiprion* (●) at 37 °C and 29 °C

Myosins were prepared from the white muscle of the two species by a rapid method[9] The time-dependent exposure of SH groups of myosin (Fig 3) was determined by measuring the increasing optical density ($\lambda = 412$ nm) of the thiophenol anion with 5,5'-dithio *bis*-2-nitrobenzoic acid (DTNB) following reaction in 0 4 M KCl, 0 1 M phosphate buffer *p*H 8 0 (refs 10 and 11) The SH groups of *Notothenia* myosin became available for interaction with DTNB very much faster than those of *Amphiprion*, the reaction time being less than 1 min for the Antarctic fish compared with approximately 15 min for the tropical species

Thus, differences in activation energy (E_a), positively correlated with adaptation temperature, may play an important role in the evolutionary temperature adaptation of the enzyme Similar differences in E_a are important factors in evolutionary rate compensation in other enzyme systems[12,13] It has also been suggested that the higher catalytic efficiencies between homologous enzyme systems from cold adapted poikilotherms compared with homoeotherms may be explained in terms of an increase in weak bond formation of the activated enzyme-substrate complex in poikilotherms[14] On this basis the num-

Fig 3 The time-dependent exposure of myosin SH groups from *Notothenia* (O) and *Amphiprion* (●) measured as the increasing absorbance (412 nm) of the thiophenol anion of DTNB at *p*H 7 0

erous weak bonds involved in stabilising the higher orders of structure of cold adapted poikilotherms necessarily make the molecule more susceptible to thermal inactivation at higher temperatures This inactivation is probably associated with the disruption of certain hydrogen bonds and hydrophobic interactions between intraprotein amino acid residues, resulting in critical changes in the tertiary structure of the protein, causing loss of enzymic activity We suggest that the higher activity of the myofibrillar ATPase of Antarctic fish at low environmental temperatures is associated with weaker bonding, in other words, with a more open molecular structure In the case of the tropical fish a more compact and rigid structure seems to be necessary to give the molecule thermal stability at higher environmental temperatures Evolutionary temperature adaptation of myosins from different fish may well have important implications in limiting the present day geographical distribution of the different species

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Metabolic and insulin-releasing activities of D-glucose anomers

GRODSKY *et al*[1] proposed that glucose stimulates insulin secretion because the sugar is metabolised in the pancreatic β cells, this view of stimulus recognition was later termed the substrate–site hypothesis[2] The hypothesis has received support from observed correlations between rates of insulin release and various parameters of glucose metabolism in the pancreatic islets It is particularly striking that a triose, D-glyceraldehyde, has been shown to mimic the electrophysiological[3] and insulin-releasing[4-6] actions of glucose, D-glyceraldehyde is oxidised and dilutes $^{14}CO_2$ arising from D-(U-^{14}C)-glucose in the islets[4-6] Although the substrate–site hypothesis has been criticised (for example, refs 7 and 8), many of the arguments raised may be doubtful for reasons discussed elsewhere[9] An important challenge to the hypothesis is the observation that the α anomer of D-glucose is more effective than the β anomer in stimulating insulin secretion[10,11] For the anomeric specificity of the D-glucose recognition system to stand up as evidence against the substrate–site hypothesis, however, it must be shown that the transport, phosphorylation or further metabolism of glucose does not exhibit the same anomeric specificity in the β cell We report here that β-D-glucose is at least as effective as α-D-glucose in stimulating counter-transport of 3-O-methyl-D-glucose,

Table 1 Effects of D-glucose anomers on the islet content of glucose-6-phosphate

Sugar	μmol glucose-6-phosphate per kg dry weight		
	Primary data	Difference from control	β minus α
None (control)	87 7±18 2	—	—
α-D-glucose 6 mM	150 7±23 3	63 0± 8 4‡	—
β-D-glucose 6 mM	242 5±41 8	154 8±27 7†	91 8±22 3*
None (control)	81 3± 6 3	—	—
α-D-glucose 18 mM	183 5±32 9	102 3±31 7*	—
β-D-glucose 18 mM	213 5±24 6	132 3±30 7*	30 0±47 9

Mean values±s e m of four different mice are given in each of two experimental series Values denote glucose-6-phosphate concentration in islets after 3 min incubation without (control) or with D-glucose anomer at the concentrations indicated In addition to data for each group of islets, the mean±s e m of differences between parallel incubations are given Levels of significance by *t* test for paired observations *$P<0$ 05, †$P<0$ 02, ‡$P<0$ 01 Two-way classification (animal/sugar) and analysis of variance of data with 6 mM anomers yielded $P<0$ 05 for α-D-glucose against control, $P<0$ 001 for β-D-glucose against control, and $P<0$ 01 for β-D-glucose against α-D-glucose

increasing the islet content of glucose-6-phosphate, and diluting $^{3}H_2O$ that results from the metabolism of D-(5-^{3}H)-glucose

Figure 1 shows that α-D-glucose (Sigma) was more effective than β-D-glucose (Sigma) in stimulating insulin release Both anomers were capable of increasing the islet content of glucose-6-phosphate, but the effect of 6 mM β-D-glucose was significantly greater than that of equimolar α-D-glucose (Table 1) When islets were incubated with D-(5-^{3}H)-glucose, the production of $^{3}H_2O$ fell on addition of either non-radioactive anomer (Table 2) In keeping with the greater effect of β-D-glucose than of α-D-glucose on glucose-6-phosphate, the effect on $^{3}H_2O$ production was statistically significant for the β anomer but not for the α anomer Neither anomer induced any measurable counter-transport of 3-O-methyl-D-glucose at 37 °C (Table 3) This lack of effect probably resulted from an extremely rapid equilibration across the β cell plasma membrane resulting in an almost immediate obliteration of any driving anomer gradient When the activity of the transport system was reduced by lowering the temperature to 8 °C, both anomers induced a significant counter-transport of 3-O-methyl-D-glucose, but there was no demonstrable difference in effect between the two

These results indicate that the anomeric preference of the insulin-releasing D-glucose recognition system is not shared by the earliest steps of glucose metabolism It follows that the discriminatory behaviour of the recognition system probably cannot be reduced to that of the glucose-transporting carrier or the glucose-phosphorylating enzymes, although direct studies of the hexokinase kinetics remain to be performed This conclusion seems to create a serious difficulty for the substrate–site hypo-

Table 2 Effects of D-glucose anomers on $^{3}H_2O$ production from D-(5-^{3}H)-glucose

Group	mmol per kg dry weight		
	Primary data	Difference from control	β minus α
Control	9 26±0 88	—	—
α-D-glucose	7 68±0 76	−1 58±1 17	—
β-D-glucose	6 62±0 95	−2 64±0 66*	−1 06±1 08

Mean values±s e m of five different mice are given Islets incubated for 10 min with 17 mM D-(5-^{3}H)-glucose in albumin-free medium produced $^{3}H_2O$ corresponding to 6 09±0 90 mmol glucose (with same specific radioactivity as in medium) per kg dry weight of islets Table shows production of $^{3}H_2O$ during same incubation followed by 5 min incubation with 17 mM D-(5-^{3}H)-glucose alone (control) or in combination with 6 mM non-radioactive D-glucose anomer As results are expressed in terms of glucose with a fixed radioactivity per mol, decreases from control values indicate dilution of the metabolically active glucose pool In addition to data recorded for each group of islets, the means±s e m for differences between parallel incubations are given
*Level of significance by *t* test for paired observations $P<0$ 02
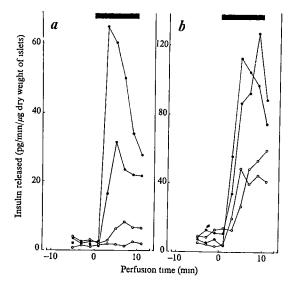
Fig. 1 Insulin release in response to D-glucose anomers Islets were perifused with glucose-free medium without (a) or with (b) 2 mM theophylline During periods indicated by bars, the medium also contained α-D-glucose or β-D-glucose In each experiment the two anomers were tested in consecutive periods separated by 30 min of perifusion with glucose-free medium In (a) α-D-glucose was tested before β-D-glucose, in (b) the order was reversed To facilitate comparisons, the periods of perifusion with glucose have been superimposed on the time scales Each point represents the average rate of insulin release in response to α-D-glucose (●) or β-D-glucose (○) over a period of 2 min The total concentration of glucose (α plus β) measured in the effluent was 9.0 ± 0.1 mM $(8.6-9.2)$ in studies of the α anomer, and 8.8 ± 0.1 mM $(8.3-9.2)$ in studies of the β anomer Two separate experiments are shown in each diagram

Fresh islets were microdissected free-hand from adult non-inbred Umeå-*ob/ob*-mice, these islets contain more than 90% β cells In most incubations the basal medium was a Krebs-Ringer bicarbonate buffer kept at 37 °C, equilibrated with ambient air, and supplemented with 20 mM N-hydroxyethylpiperazine-N¹-2-ethane sulphonic acid (HEPES) to stabilise the pH at 7.4 In

transport experiments, Krebs-Ringer bicarbonate buffer equilibrated with O_2-CO_2 (95 5) was used Unless otherwise stated, the media contained 1 mg of bovine serum albumin ml^{-1} Polarimetric observations of D-glucose anomerisation rates at 37 °C agreed with results of Niki et al[10]

To study insulin release, the perifusion apparatus described previously[12] was modified to enable solutions of glucose anomers to be injected into a stream of basal medium passing groups of 4 islets at a rate of 35 μl min^{-1} The glucose solutions were prepared and kept at 0–2 °C for 5 min before injection, the volumes injected were small enough for the temperature to equilibrate with that of the apparatus (37 °C) before glucose reached the islets about 30 s after injection Insulin was assayed radioimmunologically, and the dry weight of islets was determined as described previously[12]

To measure glucose-6-phosphate, batches of four islets were incubated for 30 min in 25 μl of basal medium only Crystals of pure anomers were quickly dissolved in basal medium and warmed for 15 s before being added to the islets, 3 min later the islets were removed and frozen After freeze-drying (−40 °C, 0.1 Pa) overnight and weighing, islets were extracted and glucose-6-phosphate was determined[13]

Production of ^3H_2O from D-(5-^3H)-glucose was measured as an index of the combined glycolytic and pentose phosphate shunt fluxes[14] Batches of three to four islets were incubated for 30–40 min in basal medium and subsequently for 10 min in 15 μl of medium containing 17 mM equilibrated D-(5-^3H)-glucose (2.0 Ci mol^{-1}) An aliquot (5 μl) of medium containing the labelled glucose alone (control) or with non-radioactive α- or β-D-glucose anomer was then added After 5 min, metabolism was stopped with 5 μl 0.27 M HCl The ^3H_2O produced was allowed to equilibrate overnight with 500 μl water that was in an enclosing outer glass vial ^3H_2O was determined by liquid-scintillation counting, and the islets were dried and weighed as above

Affinity for the D-glucose transport system was determined as the capacity of D-glucose anomers to induce counter-transport of 3-O-methyl-D-glucose at 37 °C or 8 °C After incubation in glucose-free medium for 30–40 min, batches of three islets were equilibrated with 1 mM 3-O-methyl-D-(1-^3H)-glucose (15 Ci mol^{-1}) and 0.75 mM L-(1-^{14}C)-glucose (10 Ci mol^{-1}) during incubation in 200 μl medium for 10 min After adding 2 μl of buffer, with or without the non-radioactive glucose anomer, incubation was continued for 1 or 5 min Islets were removed, freeze-dried overnight and weighed Their content of ^{14}C and ^3H was determined by liquid-scintillation counting and intracellular 3-O-methyl-D-glucose estimated by correcting for ^3H in the L-glucose (extracellular[15-17]) space

thesis, but we do not think that it is sufficient reason for abandoning that hypothesis altogether A case for caution can be made in the light of some recent experiments on anoxic islets In such islets the concentration of fructose-1,6-diphosphate does not change with glucose in the concentration range of 1–20 mM Yet, the glycolytic flux seems to depend markedly on glucose concentration in that range (B Hellman, L-Å Idahl, J Sehlin, and I-B Taljedal, unpublished) Although the rate of islet glycolysis is often assumed to be mainly controlled by glucose phosphorylation, those observations suggest the possibility of a more complex metabolic recognition system for glucose

Another reason for caution is that rejection of the substrate-site hypothesis would require a change in our explanation of glyceraldehyde-induced and dihydroxyacetone-induced insulin release The action of D-glyceraldehyde differs from that of D-glucose in not being inhibited by mannoheptulose or glucosamine[4,5] As these inhibitors probably act on the islet hexokinases, a metabolic step that is likely to be circumvented by

D-glyceraldehyde, the difference between glucose and glyceraldehyde is readily explained by the substrate-site hypothesis If one abandons this hypothesis, however, one must postulate some direct sugar receptors that will accept a few, but not many, hexoses and that will also accept trioses Those receptors must be assumed to bind mannoheptulose in such a way that the receptor's response to glucose is blocked, while that to glyceraldehyde is not Whether or not one postulates one complicated receptor common to glucose and trioses, or different receptors for glucose and mannoheptulose on the one hand and for trioses on the other, the ensuing hypothetical construct is so complicated as to be unattractive in the absence of solid evidence of a positive kind So far, results that have been interpreted as possibly reflecting properties of direct glucose receptors in the β cells may be suggestive but are far from conclusive[18,19] Only further experiments can tell whether the answer lies in a substrate-site hypothesis developed to conform with the present data, a regulatory-site hypothesis based on direct receptors, or a combination of such mechanisms[2,4]

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Table 3 Induction of 3-O-methyl-D-glucose counter-transport by D-glucose anomers

Temperature	No of experiments	mmol 3-O-methyl-D-glucose per kg dry weight		
		Control	α-D-glucose	β-D-glucose
37 °C	8	1.35 ± 0.14	1.40 ± 0.12	1.49 ± 0.18
8 °C	7	1.56 ± 0.21	$0.80\pm0.10^*$	$0.85\pm0.10^*$

Mean values $\pm$ s.e.m. are given for the numbers of mice indicated Incubations were performed at 37 °C or 8 °C Islets were equilibrated with 1 mM 3-O-methyl-D-glucose and subsequently incubated for 1 min (37 °C) or 5 min (8 °C) with or without 6 mM D-glucose anomer Table shows the intracellular content of 3-O-methyl-D-glucose at the end of incubation
*Level of significance by t test $P<0.01$

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BCG immunotherapy of rat tumours in athymic nude mice

CONSIDERABLE emphasis is now being placed on the use of bacterial adjuvants such as bacillus Calmette Guerin (BCG) and *Corynebacterium parvum* for the immunotherapy of malignant disease While general immunostimulation by these adjuvants produces a therapeutic response against some but not all experimental animal tumours[1,2], there is evidence to suggest that a more effective suppression of tumour growth can be achieved by contacting the adjuvant with the tumour[3,4] Tumour cells injected into compatible hosts in admixture with BCG often do not produce progressively growing tumours[4] and in some cases regression of established tumour grafts can be produced by intralesional injections of BCG (ref 3) or *C parvum*[5] Clinically this is akin to the observation that cutaneous melanoma nodules may regress following intralesional injection of BCG (refs 6 and 7) Adjuvant contact immunotherapy has also been used in experimental animal studies to suppress growth of tumours developing in pulmonary or pleural sites, which are models for the treatment of metastatic disease[8-10]

Host responses are involved in 'adjuvant-contact'-mediated suppression of tumour growth since the adjuvants are not directly cytotoxic for tumour cells The relative roles of specific immunity directed against tumour-associated rejection antigens and possibly less specific reactions such as those mediated by activated macrophages is not fully understood This is emphasised by studies showing that growth of several transplanted rat tumours, including a carcinogen-induced mammary carcinoma AAF57 (ref 11), could be suppressed by contact with BCG, although these tumours were inactive or only weakly immunogenic as defined by their capacity to induce immunity against

transplanted tumour cells[12] Moreover, BCG-contact suppression of transplanted rat osteosarcomas has been observed in hosts immunosuppressed by adult thymectomy and whole-body irradiation[13]

This study was carried out to investigate further the role of host immunity in BCG-mediated suppression of tumour growth by characterising the responses to transplanted rat tumours in athymic mice Several rat tumours were studied including a carcinogen-induced fibrosarcoma (Mc7) and a hepatoma (D23) both of which exhibit significant immunogenicity, as well as carcinogen-induced and spontaneously arising mammary carcinomas (Sp15, Sp22 and AAF57), which are weakly or non-immunogenic[12] The nude-athymic mice (*nu/nu*), originally described as thymus deficient (MRC Animals Centre, Carshalton), were injected subcutaneously with tumour cells either in Medium 199 or in medium containing BCG (Glaxo freeze-dried percutaneous vaccine, 500 μg moist weight organisms) The tumour cells for these tests were derived from tissue culture lines maintained in Eagle's MEM supplemented with 10% calf serum to ensure that host lymphoid cells were not transferred It has been shown, for example, that comparable to findings with rat fibrosarcomas[14], tumour cell suspensions prepared from sarcoma Mc7 and hepatoma D23 by trypsin digestion of tumour tissue contain 9–25% glass-adherent macrophages

Table 2 Growth of second challenge of sarcoma Mc7 cells in mice rejecting a mixed inoculum of Mc7 cells and BCG

First challenge			Second challenge			
Day	No of cells in admixture with BCG	Tumour takes	Day	No of cells	Tumour takes in Test	Control
0	1×10^6	0/4	0	1×10^6	2/2	—
			18	1×10^6	2/2	3/3
0	1×10^6	0/1	0	1×10^6	1/1	—

Progressive growths were produced in athymic mice with all five tumours studied using challenge inocula of between 5×10^4 and 10^6 cultured tumour cells (Table 1) When similar tumour cell challenges were administered in the presence of BCG, however, this generally resulted in complete inhibition of tumour growth With sarcoma Mc7, for example, challenges of 1×10^6 tumour cells elicited tumours in seven out of eight mice in three separate experiments, whereas only one mouse receiving Mc7 cells together with BCG developed a tumour BCG treatment also prevented growth of 2×10^5 mammary carcinoma Sp15 and 8×10^4 mammary carcinoma Sp22 cells, although larger tumour cell challenges were not suppressed None of the tests, however, clearly established suppression of tumour AAF57 by contacting with BCG

Previous studies[4,15] in immunocompetent rats with sarcoma Mc7 showed that rejection of tumour cell/BCG mixed inocula produced a specific immune response and this induced rejection of tumour developing at a contralateral site In this case, rejection was mediated by a specific response against tumour-associated antigens since challenge with another tumour expressing different neoantigens was not rejected Comparable tests in the athymic mouse model indicate that suppression of sarcoma Mc7 by contact with BCG does not produce inhibition of a challenge with the same tumour either injected simultaneously at a contralateral site or given subsequently after rejection of the tumour cell/BCG inoculum (Table 2)

The implication from these studies is that suppression of tumour growth is not effected by a T lymphocyte mediated response, although the characteristics of the effector mechanisms are still to be elucidated These may involve a humoral antibody although previous attempts to suppress growth of sarcoma Mc7 with tumour-immune serum in immunocompetent rats have mostly been unsuccessful There is stronger evidence, however, to suggest that the effects induced by adjuvants such as BCG may be mediated by macrophages as tumour suppression is abrogated in silica-treated rats (R W B , and D G Hopper, unpublished[8]) Of more practical significance is the observation

Table 1 BCG-mediated suppression of rat tumours in athymic mice

Tumour	No of cells injected in admixture with BCG*	Tumour takes in Test	Control
Sarcoma	1×10^6	0/2†	2/2
Mc7	1×10^6	0/2	2/2
	1×10^6	1/4	4/4
Hepatoma	1×10^5	1/3	3/3
D23	1×10^5	0/3	3/3
Mammary carcinoma Sp15	2×10^5	0/3	3/3
	1×10^6	3/3	2/2
Mammary carcinoma Sp22	8×10^4	0/2	2/2
Mammary carcinoma	1×10^4	0/3	0/3
	5×10^4	1/3	2/3
AAF57	1×10^5	2/2	2/2

*Glaxo percutaneous vaccine (500 μg moist weight BCG organisms)
†None out of two, and so on

that suppression of tumour growth following contact with adjuvants such as BCG can be achieved in hosts in which immunocompetence is impaired This may have considerable bearing on the development of clinical studies where immuno-therapeutic approaches are often attempted in patients where immunosuppression is already evident as a consequence of surgery, radiotherapy and/or chemotherapy

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Ir-gene control of immunogenicity of insulin and A-chain loop as a carrier determinant

Ir is generally believed that the B cells produce antibody against certain antigens after the helper cells recognise a determinant on the carrier part of the antigen The immune response in mice against several antigens is controlled by Ir-genes which map in the major histocompatibility (H-2) locus of the IXth mouse linkage group Details of the function of the Ir-genes are still unknown, but it is generally accepted that they control the recognition of carrier determinants[1,2]

Although several different antigens are known whose recognition is Ir-gene dependent, the specificity of this recognition mechanism as well as the structure of the carrier determinants remains unclear

To evaluate the specificity of Ir-gene dependent recognition it is necessary to have a carrier molecule with the following properties (1) It should be available in highly purified form (2) Its tertiary structure must be known (3) It should bear only one carrier determinant (4) A homologous molecule with small differences at a definite site must be available, in which these alterations do not change the remaining structure of the molecule (5) Only one of the two homologous molecules must be immunogenic in the strain under investigation

A comparison of the structure of the two molecules would

yield information about structural requirements for a carrie determinant and about the specificity of the Ir-depender carrier recognition

The insulin molecule satisfies all these requirements It is small molecule (molecular weight=5,750) so that the number c carrier determinants should be limited for steric reason: Since the mice tested have their own insulin a further restrictio of determinants may be expected Insulins of different specie show small differences in sequence, though the overall structur of the molecule is not altered The immune response against these carriers is Ir-dependent (unpublished results)

In these experiments the dinitrophenyl(DNP)-derivative c insulin with one DNP-group at lysine in position 29 of th B chain has been used This derivative was chosen because i can be isolated in a highly purified form by electrofocusing[3] hence it can be regarded as free from other contaminating substances which might be highly immunogenic[4,5] Another advantage is that the DNP-group as a haptenic determinan
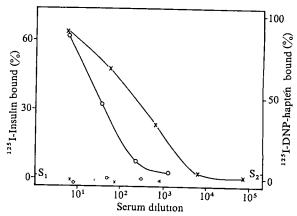
Fig 1 Response of C57BL mice against DNP-lysine[B29] insulins ×, Anti-insulin response, O, anti-DNP response Pooled sera were tested Mice were injected with DNP-lysine[B29] bovine insulin (solid line) or DNP-lysine[B29] pig insulin (dotted line) Anti-DNP antibody was measured by the Farr-test using ^{125}I-α,N-(4-hydroxyphenacetyl)-ε-N-2,4-dinitrophenyl-L-lysine as hapten[11] Anti-insulin antibody was assayed with ^{125}I-bovine insulin by precipitation with 10% polyethyleneglycol[12] The antibody binding capacity (ABC) of a high titre guinea pig antiserum was set as 100% S_1, S_2, standard deviations of background Mice were injected with 20 µg antigen on day 1, boosted on day 21 with the same dose in saline and bled on day 30

serves as an internal control for the processing of the antigen by the antibody-producing B cells The response pattern is not affected by the introduction of the DNP-group, it also applies to non-substituted insulin, highly purified by electrofocusing, see Table 1

If pig insulin is used as a carrier the mouse strain BALB/c (H-2[d]) gives a high titre of anti-insulin and anti-DNP-antibody, whereas the strains C57BL (H-2[b]) and C57BR (H-2[k]) show no immune response at all (Fig 1 and Table 1) If bovine insulin

Table 1 Response of different mouse strains against pig and bovine insulins*

Strain	H-2	Immunogen	Anti-insulin antibody		Anti-DNP antibody	
BALB/c	d	DNP-Lys[B29] pig insulin	3 61	(1 22)	3 89	(1 19)
C57BL	b	DNP-Lys[B29] pig insulin	<0 1		<0 1	
C57BR	k	DNP-Lys[B29] pig insulin	<0 1		<0 1	
BALB/c	d	DNP-Lys[B29] bovine insulin	4 51	(1 24)	3 18	(1 22)
C57BL	b	DNP-Lys[B29] bovine insulin	3 08	(1 20)	2 7	(1 26)
C57BR	k	DNP-Lys[B29] bovine insulin	<0 1		<0 1	
B10 BR	k	DNP-Lys[B29] bovine insulin	<0 1		<0 1	
C57BL	b	Pig insulin	<0 1		—	
C57BL	b	Bovine insulin	3 45	(1 31)	—	

*Logarithmic mean of individual titres, s d coefficient given in parentheses Each group 7-10 mice Experimental details same as in Fig 1

is used as carrier, C57BL (H-2^b) is a responder, whereas C57BR (H-2y) is a non-responder with both insulins. The non-response of B10.BR (H-2^k) is good evidence for the Ir-control of the immune response, since this strain is congenic with C57BL, the only difference being at the H-2 locus.

BALB/c mice (H-2^d) respond to both insulins, so they obviously recognise different determinants, which are not recognised by C57BL mice. The non-response of C57BL against DNP-Lys^{B29} pig insulin may be caused by a deficiency of B cells which can respond to the pig insulin determinants but this is improbable as it has been found that (1) C57BL cannot use pig insulin as carrier for an anti-DNP response and (2) C57BL antibodies against bovine insulin do cross react with pig insulin. The only difference between the two insulins (Fig. 2) is in two amino acids at positions A$_8$ and A$_{10}$ in the A chain. These amino acids are located in a small loop which is formed by six amino acids (A$_6$ to A$_{11}$) and a disulphide bridge. The sequence of this loop is the same in pig and mouse insulin, whereas bovine insulin contains alanine instead of the more hydrophilic threonine at A$_8$, and valine instead of isoleucine at A$_{10}$. The simplest explanation is that the A-chain loop of bovine insulin is the carrier determinant which is recognised by H-2^b mice.

Pig-Insulin
Mouse-Insulin

Bovine-Insulin
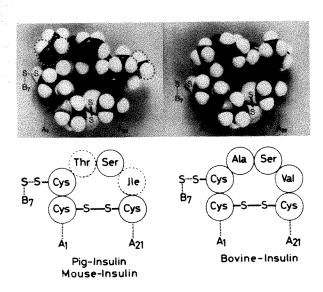
Fig. 2 Structure of the A-chain loop of pig and mouse insulin (left side) which does not act as carrier determinant and the homologous structure (right side) of bovine insulin which acts as carrier determinant in C57BL mice. In the upper picture the marked areas in the 'non-determinant' structure show the –CH(OH)– and the –CH$_3$ group which are lacking in the determinant.

It may be argued that the exchange of these two amino acids leads to a change in the tertiary structure of the whole molecule, so that the true determinant may not be the A-chain loop itself but another site on the molecule. But this seems to me to be highly unlikely, as this loop is a very rigid structure on the surface of the molecule, further strengthened by a second disulphide bridge at A$_7$, which links the A chain to the B chain. Therefore, it seems unlikely that changes in this loop affect the overall structure of the molecule[6]. Furthermore there is no difference in biological activity between both insulins.

Our experiments do not show whether the size of the carrier determinant is exactly that of the A-chain loop. Some adjacent parts of the molecule may be necessary or only a part of the loop may act as carrier determinant. It is also possible that, in addition to the loop structure, other sites on the molecule have to be recognised as carrier determinants. In that case, however, their contribution alone would not be sufficient for induction which we have shown in C57BL (H-2^b) mice to be dependent on the presence of the bovine A-chain loop.

It is obvious that the differences between the 'non-determinant' structure (A-chain loop of pig insulin) and the determinant (A-chain loop of bovine insulin) are rather small. There is no charge difference in the two cases and the size and steric arrangement of both structures are very similar. It seems clear that the recognition unit which can discriminate between these two structures must have a high degree of specificity.

It is of special interest to ask whether the specificity controlled by the Ir-genes is reflected in the antibody population, as has been reported[7]. If the sera shown in Table 1 are absorbed with pig insulin coupled with Sepharose, no anti-insulin activity is left in any of them (our unpublished results). Thus, it seems that the high degree of discrimination at the carrier level is not reflected at the level of antibody production, and conversely that the specificity of the antibody is not reflected at the carrier level in C57BL mice. A somewhat similar situation has been described for the hormone glucagon[8].

Finally, the finding of carrier determinants on insulin and Ir-dependence of their recognition adds same interesting aspects to the problem of insulin therapy in diabetics. Some recent papers claim that pig insulin is non-immunogenic in rabbits if highly purified[4,5]; others show the opposite[9]. It seems very likely that these discrepancies result from the different genetic background of the experimental animals.

As the insulins of man, mouse and pig have the same sequence in the A-chain loop it seems very likely that the bovine insulin loop can also act as a carrier determinant in man. This assumption is confirmed by the high incidence of anti-insulin antibody in patients treated with the commonly used bovine insulin compared with those treated with pig insulin. It seems reasonable to assume that in man, too, an Ir-gene controls the recognition of this determinant. Until now there has been only weak evidence that such an Ir-gene is located in the HL-A locus of man which corresponds to the H-2 locus in the mouse[10]. Experiments designed to establish a correlation of the ability to make antibody against bovine insulin with a certain configuration in the HL-A locus would provide evidence for this assumption.

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Function of peptide antibiotics in producer organisms

PEPTIDE antibiotics in the producer organisms have been ascribed a wide variety of roles, ranging from evolutionary relics and waste products to elevated control in metabolism (see refs 1 and 2). No hypothesis, however, has yet found

general acceptance. A frequent suggestion is that these antibiotics may have an important regulatory function during the sporulation process of the producer cells[3,4]. We have recently reported, however, the existence of a bacitracin-negative mutant of *Bacillus licheniformis* which sporulates normally[5], thus ruling out a direct role for the peptide antibiotic in the formation of spores in this species. To find out whether this mutant is a true non-producer of bacitracin or not, we have compared the bacitracin synthetase complex in this mutant with that of the bacitracin-producing mother strain *B. licheniformis* AL. This suggests that the mutant is not able to produce any bacitracin as a result of a defective enzyme complex.

The non-ribosomal synthesis of the peptide antibiotic bacitracin[6-8] is in principle similar to the protein thiotemplate mechanism[9] for the synthesis of gramicidin S and tyrocidine. The purified enzyme complex from the bacitracin producer *B. licheniformis* AL was resolved in three complementary fractions (A, B, and C) using affinity chromatography. The chromatogram (Fig. 1a) was nearly identical to that obtained using bacitracin synthetase from *B. licheniformis*, ATCC10716 (ref. 10). Each of the three enzyme components activated a different group of the amino acids (measured by the $^{32}PP_i$–ATP exchange reaction) which make up the bacitracin molecules. All three components were necessary for the bacitracin synthesis. Any exchange of corresponding enzyme components

Table 1 *In vitro* synthesis of bacitracin by combined bacitracin synthetase components

Enzyme component	Radioactive incorporation in bacitracin spot (c.p.m.)
A	85
B	120
C	138
AC_0	115
B_0	83
A+C	94
A+C+B_0	1010
B+C	190
AC_0+B+C	155
A+B+C	795
AC_0+B_0	90

Bacitracin synthetase from *B. licheniformis* AL and SB319 was fractionated by affinity chromatography as described in Fig. 1. A slower increasing gradient gave the following cross contamination of the fractions A, B, C, AC_0 and B_0 after precipitation with solid $(NH_4)_2SO_4$ (to 80% saturation) and carrier BSA: Component A contained 8% C; B—4% C; C—15% A; AC_0—3% B_0; and B_0—3% AC_0. The enzyme components were combined (20 µl of each fraction), and the bacitracin synthesising activity was measured (after 15 min incubation at 37°C) using ^{14}C-L-isoleucine (85,000 c.p.m., specific activity 100 Ci M^{-1}) as described[10]. As a result of the experimental separation techniques[8] a certain background count was observed.

from strain AL and strain ATCC10716 did not reduce the amount of bacitracin produced (data not shown).

The chromatogram obtained by fractionating the bacitracin synthetase of the bacitracin-negative mutant *B. licheniformis* SB 319 was different. Using the same affinity chromatography column, the enzyme complex was resolved into two fractions (AC_0, and B_0). Fraction AC_0 activated the same amino acids as the enzyme components A and C of the mother strain, and fraction B_0 activated the same amino acids as enzyme component B (Fig. 1b). There was a marked difference, however, in the degree of the $^{32}PP_i$–ATP exchange reaction with enzymes from the producer and the non-producer. When the non-producer was collected later in growth, the enzyme component AC_0 seemed to be arbitrarily split up. The activation of L-glutamic acid was performed by a component dissociated from AC_0.

The combination of enzyme fractions from the producer

and the non-producer showed that enzyme component B of the non-producer was able to participate in the bacitracin synthesis of the producer strain instead of enzyme componer B, but enzyme component AC_0, when combined with intact enzyme component B from the producer, was not able to do s (Table 1). Similar results were obtained when enzyme components A, B, and C from ATCC10716 were used. This sugges that the bacitracin synthetase of the bacitracin-negativ SB319 was defective. This defect seems to influence th activation sites for the amino acids and the affinity between th
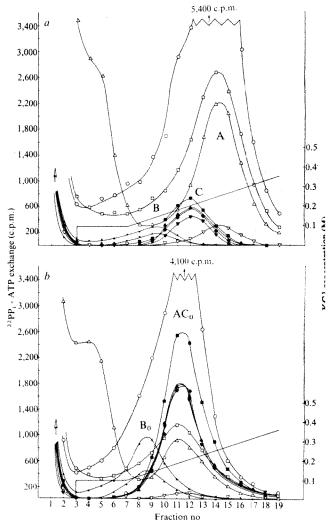
Fig. 1 Fractionation of bacitracin synthetase by affinity chromatography. *B. licheniformis* AL (*a*) and SB319 (*b*) were grown in the medium of Haavik and Thomassen[5]. The cells were collected at an absorbance of 4.5 measured in a Spectronic 20 spectrophotometer at E_{650}, lysed with lysozyme, and the enzyme was partially purified by streptomycin sulphate precipitation and ammonium sulphate precipitation (to 55% saturation) as described by Fröyshov and Laland[8]. The 55% $(NH_4)_2SO_4$ fraction obtained from 250 ml culture was applied to an affinity chromatography column (3.0 × 1.6 cm) prepared from 2 g Sepharose 4-B (Pharmacia Fine), and 3, 3′-diamino-dipropylamine, with L-leucine as ligand as described[8]. The column was eluted with 0.05 M potassium phosphate buffer (*p*H 7.5) containing 0.2 M $MgCl_2$ and 0.1 mM dithiothreiol (60 ml h^{-1}). A KCl gradient (0.1–0.4 M) was added to the buffer, and $^{32}PP_i$–ATP exchange activity dependent on the L-amino acids isoleucine (○), cysteine (□), leucine (△), glutamic acid (▽), lycine (+), ornithine (×), phenylalanine (▼), histidine (■), aspartic acid (▲), and asparagine (♦) was measured in 0.2 ml aliquots of each fraction (6.5 ml) as described[10]. Total amount of radioactivity in each incubation mixture was 120,000 c.p.m. The radioactivity (40–50 c.p.m.) in control tubes (without any amino aicds) was subtracted. The signs A, B, C, AC_0, and B_0 indicate the enzyme fractions used in further experimentation.

enzyme components corresponding to A and C of the producer

Previously[5], neither external nor internal bacitracin were detected yet the mutant SB319 sporulated normally, indicating that bacitracin has no regulatory role during sporulation With reports of other antibiotic-negative mutants able to sporulate[11,12], evidence seems to be accumulating against a possible connection between peptide antibiotics and sporulation of producer organisms
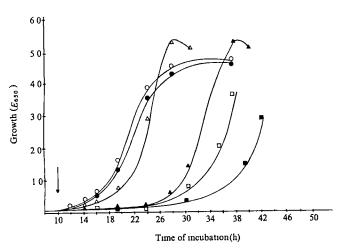
Fig 2 Growth of *B lichemformis* strains at different cultural conditions Growth of strain SB319 in the medium with 0 0001 g $MnSO_4l^{-1}$ (O), with 0 1 g $MnSO_4$ l^{-1} (△), with 0 1 g $MnSO_4$ l^{-1} and 50 IU bacitracin ml^{-1} added (□) Arrow indicates time of addition The corresponding growth curves of strain AL are marked ●, ▲, ■, respectively Growth was measured as E_{650} using a Specronic 20 spectrophotometer Growth occurred in 500 ml Erlenmeyer flasks incubated at 37 °C at about 360 r p m on a New Brunswick rotatory shaker (Model G–53) The defined medium used had the following composition (g l^{-1}) L-glutamic acid (15 0), L-leucine (2 0), L-histidine (0 2), L-alanine (0 2), citric acid (1 0), KH_2PO_4 (0 1), K_2HPO_4 (0 1), $MgSO_4$ $7H_2O$ (0 2), $FeSO_4$ (0 01), $MnSO_4$ H_2O (varied between 0 0001 (low) and 0 1 (high) as noted in the text), pH 7 0 The inoculum consisted of spores[13]

The production of peptide antibiotics after growth is assumed to be a fundamental property of the microbial cell, and thus the search for their function has been focused on the stationary phase metabolism[2-4] We have reported, however, that the lag in antibiotic production in relation to growth may be pH-dependent[13,14] Peptide antibiotics may therefore normally be synthesised, and may also function throughout active growth The observations that tyrocidine and linear gramicidin production under certain conditions are paralleling growth[15], that polymyxin E production occurs in the log phase of growth[16], and that penicillin[17], tyrothricin[18], and gramicidin S (ref 19) can be produced in continuous culture, support this view

Both the producer and the non-producer strains of *B lichemformis* show similar sporulation and also identical reactions in standard biochemical tests[5] To determine the function of bacitracin during growth, the two strains were grown at several different cultural conditions At present only a few significant differences between the producer and the non-producer have been discovered The bacitracin-negative mutant showed a markedly increased resistance to high concentrations of Mn^{2+} In media with low manganese both strains showed almost identical growth (Fig 2)

Active manganese transport in *B subtilis* has recently been described Too high concentrations of manganese in the medium inhibited growth as a result of an efficient uptake of the ion[20,21] Our bacitracin-negative mutant, SB319, was only slightly inhibited at manganese concentrations which strongly inhibited the producer strain AL, indicating that the manganese uptake of the mutant was markedly less efficient than that of the producer If bacitracin participates in the transport of

Mn^{2+}, this difference between the two strains could be explained Furthermore, the addition of bacitracin to the bacitracin-negative strain should eliminate this difference Indeed, when bacitracin was added to the non-producer, growth was markedly inhibited in media with high manganese (Fig 2) Adding bacitracin to the same culture grown in low manganese showed no effect The addition of bacitracin to the producer strain increased the inhibitory effect of high manganese concentration (Fig 2)

The possibility that peptide antibiotics normally act as carriers of ions through membranes in the producer organisms has been suggested[22,23] This agrees with the strong metal binding properties of peptide antibiotics[24,25], and their ability to modify membrane permeability[26] Since the bacitracin-negative mutant, SB319, is able to grow without producing any bacitracin, this mutant must take up manganese by some other means Our results indicate, however, that bacitracin is able to influence this manganese transport by increasing the efficiency of the uptake of the ion By depriving the non-producer and the producer strains of manganese by adding EDTA to the medium, the bacitracin-negative mutant was markedly inhibited whereas the bacitracin producer grew well By adding bacitracin or excess manganese to the EDTA-inhibited mutant, growth was promoted (H I H, unpublished) This indicates that bacitracin production may be necessary in environments with a very low manganese content

The antimicrobial effect of bacitracin may be an interaction with the cytoplasmic membrane of susceptible organisms[26] This effect is dependent on the presence of certain divalent cations such as Mn^{2+} (ref 27), the function of which may be to promote the cell–antibiotic interaction[26] A similar mechanism may operate in the producer strain bacitracin may increase the uptake of manganese by promoting the interaction between manganese and the cytoplasmic membrane

In conclusion, we have shown that the bacitracin-negative mutant (SB319) has only a partially functional bacitracin synthetase complex This mutant is probably not able to produce any molecules of bacitracin A comparison of the producer and non-producer of bacitracin in different cultural conditions indicates that the peptide antibiotic bacitracin participates in the transport of manganese through the bacterial membrane Further experimentation will be necessary to determine whether this is causal or coincidental

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Improvement of antisera against polypeptide hormones

THE sensitivity of immunoassays is probably decreased by endogenous antigenic material blocking some of the antibody binding sites[1]. To remove antibodies generated against polypeptide hormones from endogenous antigenic material derived from the immunised animals, we took advantage of the capacity of sodium iodide to break the antigen–antibody bond

Antisera against lys_8–vasopressin (Ferring, Malmo) and val_5–angiotensin II (Ciba, Basel) were generated in rabbits with repeated intramuscular injections of immunogen coupled to albumin[2] Angiotensin was labelled with [125]I (IMS 3, Amersham) as described previously[2] In the case of arg_8–vasopressin (Ferring, Malmo) the metabisulphite step was omitted Only monoiodinated material was used, as confirmed by isoelectric focusing Specific activity for angiotensin II was 450 μCi μg^{-1}, and for arg_8–vasopressin 650 μCi μg^{-1}

Antisera were diluted at ratios of 1 700–1 2,000 with 0 9% NaCl and treated with 2M NaI (final concentration) at room temperature for 2 h, then dialysed for 48 h at +4 °C against
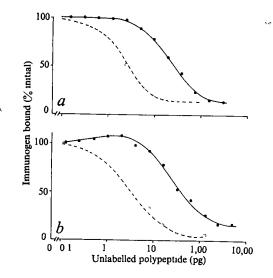
Fig. 1 Increase in sensitivity *a*, Dose–response curve for val_5–angiotensin II (mean of five experiments) before (●) and after (○) treatment of the antiserum with NaI *b*, Dose response of arg_8–vasopressin (mean of eight experiments) shows a paradoxical increase in binding[5,6] before (●) and a shift to the left with disappearance of the paradoxical binding after (○) iodide treatment

0 9% NaCl The diluent for binding studies with vasopressin was 0 15 M phosphate buffer (*p*H 7 5), containing human serum albumin (Kabi, Stockholm) 1 mg ml^{-1}, Na_2–EDTA 10 mM, L–cysteine 20 mM neomycin sulphate (Upjohn) 0.1 mM, and for angiotensin the same buffer with OH–quinoline 1.5 mM (Merck) instead of L–cysteine

The procedure of sodium iodide treatment and subsequent
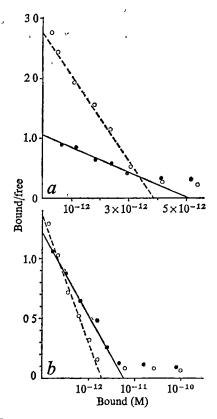
Fig 2 Increase in affinity Scatchard plots[4] for antisera to angiotensin II (*a*) and lys_8–vasopressin (*b*) before (●) and after (○) NaI treatment (mean of three experiments) The apparent affinity constants[3] were 2 1 × 10^{-13} M^{-1} and 7 1 × 10^{-13} M^{-1}, respectively, for untreated and treated antiserum to lys_8–vasopressin, and 1 7 × 10^{-13} M^{-1} and 5 9 × 10^{-13} M^{-1} respectively, for angiotensin II

dialysis increased the sensitivity of the dose–response curves for arg_8–vasopressin and angiotensin II by factors of 12 1 and 10, respectively (Fig 1) This increase in sensitivity could not be explained as a dilution effect because conditions were designed to give maximal sensitivity[3] both before and after NaI treatment of the antiserum Scatchard plots[4] (Fig 2) indicated modification of the apparent affinity constants of the antisera with iodide treatment, and some loss of total binding capacity We conclude that this resulted from the unmasking of high affinity antibody binding sites blocked by endogenous antigenic material

The dose–response curves for unlabelled analogues of angiotensin II and vasopressin, taken as a measure of specificity, were shifted (Fig 3) This resulted in an increased specificity for val_5–angiotensin II compared with the 2–8 heptapeptide and 3–8 hexapeptide fragments In the vasopressin assay system, iodide treatment of the antiserum caused the highest increase in sensitivity for unlabelled lys_8–vasopressin compared with arg_8–vasopressin and pressinoic acid In other words, the assay became more sensitive to the hapten (lys_8–vasopressin) actually used to immunise rabbits against vasopressin We believe that the changes in apparent specificity observed resulted from the unmasking of binding sites with a high affinity for this molecule

The paradoxical binding of vasopressin (that is, increase in bound label with low doses of unlabelled polypeptide), which has been used by others in highly sensitive radioimmunoassays for ACTH–(ref 5) and LH–releasing hormone[6], disappeared with sodium iodide treatment (Fig 1) Thus, paradoxical binding may be caused by endogenous antigen interfering with the binding of iodinated polypeptide On the other hand, iodide treatment may induce changes in the steric configuration of antibody molecules resulting in a loss of paradoxical binding

Our data suggest that any procedure for removal of endogen-
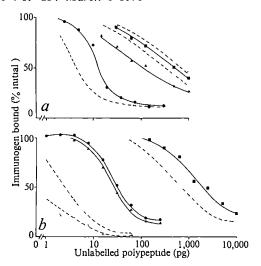
Fig 3 Modification of specificity a, Dose response curve of val_5-angiotensin II (●) radioimmunoassay is shifted to the left (○), curves of 2–8 $ileu_5$-heptapeptide (▲) and 3–8 $ileu_5$-hexapeptide (■) are shifted to the right (△, □) after sodium treatment, resulting in a decreased cross reaction with heptapeptide and hexapeptide Mean of two experiments b, Curves of lys_8-vasopressin (●), arg_8-vasopressin (▲) and pressinoic acid (Ferring, Malmo, ■) were shifted to the left after iodide treatment (○, △, □) The result was increased sensitivity for lys_8-vasopressin compared with arg_8-vasopressin and a decreased cross reaction with pressinoic acid after iodide treatment Mean of three experiments

ons antigenic material which causes minimal damage to the antibodies should be considered when adequate sensitivity of immunoassays cannot be achieved with known designs of competitive protein binding assay[3] The treatment of antisera with chaotropic substances such as sodium iodide may also result in a modification of antiserum specificity

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Conservation of primary structure in 16S ribosomal RNA

THERE is no doubt that the large ribosomal RNAs play specific roles in ribosome function[1] Yet the thrust of experimentation during the past 5 years indicates clearly that the biologist tends to view these roles as structural, function being reserved by and large for the ribosomal protein components[2,3] Whether or not this prejudice can be maintained remains to be seen In any case, the existence of an approximate sequence for a 16S rRNA provides a basis for detailed characterisations of this molecule[3,4] Here we begin to define 16S rRNA function by localising the major conserved regions in the molecule through a comparative analysis of 27 prokaryotic 16S rRNA primary structures

Comparative sequencing has been used effectively to

investigate the structure and function of a number of small macromolecules Although this approach is not feasible for the larger ribosomal RNAs, partial sequencing techniques can be used effectively One can, for example, characterise a large RNA by comparative cataloguing[5,6], that is, completely delineate the products of T_1 ribonuclease digests of RNA species from various organisms In practice, this can be done using present techniques[7] with only a few oligonucleotides eluding sequencing

When comparing two such oligomer catalogues, a prime consideration is the size for which oligomer sequence coincidence implies true primary structural homology Statistical considerations[5] indicate that coincidence among oligonucleotides of length six or more (pentanucleotides are marginal) provides strong evidence for primary structural homology in a sequence of 1,600 nucleotides When three or more catalogues are considered, the continuous appearance of any oligonucleotide of length five or more increases the probability of true homology, as the prospect of multiple chance coincidence shrinks rapidly as the number of catalogues considered increases

During the past few years, complete ribonuclease T_1 catalogues have been developed in our laboratory for the 16S rRNAs of a wide range of prokaryotes, including Enterbacteriaceae[8–10], Spirillaceae[10], Cyanophyta[11] and Athiorhodaceae[12], and there are unpublished results from this laboratory for Bacillaceae, Brucellaceae, Achromobacteraceae, Pseudomonadaceae and Lactobacillaceae It is now appropriate to consider the extent to which each of the characteristic oligomers (pentanucleotides and larger) of *Escherichia coli* 16S rRNA is conserved across phylogenetic lines These 114 oligonucleotides are shown in Table 1 together with the number of organisms in which each has been found They are arranged in their reported order of occurrence[4] in the 16S rRNA sequence and numbered alternately so that nomenclature adjustments for minor sequence revisions can be accommodated readily

When the relative occurrence data of Table 1 are depicted graphically (Fig 1), the pattern of conservation characterising 16S rRNA primary structure is displayed vividly The oligomers that appear in more than half the organisms screened are considered to be conserved, while those found in at least 24 of the 27 organisms are termed universal Figure 1 suggests nine possible concentrations of conserved and universal oligomers (1) oligomers 217–223, (2) 185–207, (3) 167–177, (4) 153–159, (5) 127–137, (6) 107–113, (7) 75–83, (8) 41–51 and (9) 31–35 Although the conserved oligomers are divided evenly between the two halves of the sequence, the universals are not (19 out of 27 are in the 3' half) Thus, as might be expected, six of the nine highly conserved regions are in the 3' half of the molecule Indeed, the 3' half of the molecule seems to alternate quite regularly between regions of extremely high and low conservation

The most prominent region of conservation, No 2, is very close to the 3' end of the molecule, between oligomers 185 and 207 The 12 large oligomers in this region together encompass approximately 100 nucleotides The final oligomer in this grouping, $U\overset{*}{C}ACACCAUG$, is present in only 14 organisms The clearly related sequences, $UCA\overset{*}{C}ACCAUG$ and $UCA\overset{*}{C}ACCACG$ (asterisks indicate post-transcriptionally modified nucleotides), however, account for 12 of the remaining organisms

Several additional regions deserve further comment Region No 1, the 3' terminal region, is excised intact by colicin E3 (refs 13 and 14) In a large group of prokaryotes, this region contains the kasugamycin-sensitive sequence[15] which may provide a clue to the region's functional significance Region No 6 comprises merely three oligomers but may be somewhat larger as it lies immediately adjacent to a gap in the sequence Three oligomers not currently placed that might appear in this gap are the highly conserved CAAACAG and the possibly universal copies of two pentamers, AUUAG and AAAUG (Table 1 and footnotes a and k)

In addition to locating the conserved regions, it is important

Table 1   Phylogenetic conservation of oligonucleotides in E coli 16S ribosomal RNA

	Oligomer composition	No of occurrences	Comments		Oligomer composition	No of occurrences	Comments
1	pAAAUUG	4		115	ACUUG	7	
3	AUCAUG	12	B	117	CCCUUG	5	
5	CUCAG	26/8	Two copies	119	CCUUCG	15/10	Two copies
7	CCUAACACAUG	11		121 (g)	CUAACG	19	
9	UAACAG	7	B	123	UUAAG	26/13/4	Three copies
11	CUUUG	4		125 (h)	ACCCG	8	
13	UAAUG	17		127	UUAAAACUCAAAUG	14	
15	AAACUG	21		129	AAUUG	26	
17	AUAACUACUG	10		131	CACAAG	27	
19	AAACG	13		133	AUG*	11	
21	CUAAUACCG	21		135	CAACG	26	
23	CAUAACG	3		137	UUUAAUUCG	25	
25	ACCUUCG	7		139	AACCUUACCUG	3	
27	CCUCUUG	3		141	UCUUG	18	May be two copies
29	CCAUCG	3		143 (i)	ACCAUCACG	0	
31	CCCAG	20		145	UUUUCAG	1	
33 (a)	AUUAG	25/14	Two copies, B in one case	147	CUUCCG	6	
35	UAACG	17		149	AACCG	8	
37	CUCACCUAG	5		151 (j)	UCCCG	27	Second copy, if prese may be here No 151 not shown on Fig A, B, C
39	AUCCCUAG	7					
41 (b)	ACCAG	6/0	May be two copies				
43	CCACACUG	24		153 (k)	AAAUG	25/12	A, B, C
45 (c)	AACUG	17/12	Multiple copies at least 2, possibly 3 may be two copies	155	UUAAG	26/13/4	A, B, C
				157 (j)	UCCCG	27	B
47 (b)	ACACG	27/4		159	C*AACG	27	
49	UCCAG	10		161	CAACCCUUAUCCUUUG	6	
51	ACUCCUACG	23		163	AACUCAAAG	5	
53	AAUAUUG	14		165	AUAAACUG	3	B
55	CACAAUG	10		167	UCAAG	21	
57	CCAUG	14		169	UCAUCAUG	14	
59	CCUUCG	15/10	Two copies	171 (l)	CCCUUACG	14	
61	UAAAG	27/17	Multiple copies, at least 2, possibly 3	173 (b)	ACCAG	6/0	May be two copies
63	UUAAUACUUUG	2		175 (m)	CUACACACG	22	
65	CUCAUUG	1		177	CUACAAUG	26	
67	UUACCCG	3		179	CAUACAAAG	2	
69 (d)	CCACG or CACCG	13/16		181	ACCUCG	4	
71	UACUUUCAG	6		183	ACCUCAUAAAG	4	
73	CUAACUCCG	12		185	CAACUCG	25	
75 (e)	G*CCG	27		187	ACUCCAUG	8	
77	UAAUACG	25	B	189	AAUCG	24	A, B
79	UUAAUCG	13		191	UAAUCG	27	B
81	AAUUACUG	14		193	AUCAG	26	
83	UAAAG	27/17	Multiple copies, at least 2, possibly 3 three copies	195 (d)	CACCG or CCACG	16/13	
				197	AAUACG	26	
85	UUAAG	26/13/4		199	UUCCCG	26	
87	AAAUCCCCG	6		201	CCUUG	23	C
89	CUCAACCUG	6		203	UACACACCG	25	B, C
91 (c)	AACUG	17/12	Two copies	205 (n)	CC*CCG	27	
93	CAUCUG	3		207	UC*ACACCAUC	14	
95	AUACUG	6		209	AUUCAUG	9	A
97	UCUCG	3		211	CAAAG	9	A
99	AAUUCCAG	5	B	213	CUUAACCUUCG	8	A
101	AUCUG	13	B	215	CUUACCACUUUG	6	A
103	AAUACCG	11		217	U*AACAAG	27	A, C
105 (f)	CCCCCUG	11		219 (g)	UAACCG	19	
107	CUCAG	26/8	Two copies	221	A*A*CCUG	14	A, B
109	AUACCCUG	26	A, B	223 (o)	AUCACCU (U, CC) UA_OH	13	A
111	UCCACG	22	B	(p)	CAAACAG	23	
113	UAAACG	26	B	(p)	ACCAAAG	6	

The first column lists the 114 ribonuclease T_1 oligonucleotides ($\geqslant$ pentamers) in the order in which they have been placed by Ehresmann *et al* in th 16S rRNA sequence unless indicated otherwise in the footnotes[4] The oligomer sequences used, however, are those determined by Uchida *et al* rather than those reported by Fellner *et al* [17], unless otherwise indicated in the footnotes The second column indicates the number of organisms in whic the given oligomer has been found The final column indicates by (A) those oligomers which have been found to be excised by ribonuclease T_1 fron intact 30S subunits (T U and C R W, unpublished), by (B) those oligomers which have been found to be substituted by kethoxal at the 5' guanosin and by (C) those substituted at the 3' guanosine[16]

Notes to Table 1

(a) This oligomer is found in two copies in E coli and most 16S rRNAs from other Enterobacteriaceae and Spirillaceae, but in one copy in many other organisms Since Ehresmann places only one copy, it is not clear that position 33 is the correct location for the universal copy If this universal oligomer is ultimately placed elsewhere, conserved region No 9 will no longer exist

(b) Ehresmann locates two copies of ACCAG (No 41 and No 173), one copy of ACACG (No 47) and no copies of CACAG Previously Fellner reported one of each Uchida found 1–2 copies of ACACG, only one copy of ACCAG and no CACAG Thus these three oligomers are currently ambiguous and are treated as such on Fig 1

(c) This oligomer has been reported as present in 2–3 copies by Uchida and 3 copies by Fellner Yet only two have been located by Ehresmann (No 45 and No 91) and one of these (No 91) is considered to be fractional

(d) Due to the inability of Fellner and Ehresmann to distinguish between CACCG and CCACG, these two oligomers (No 69 and No 195) are not uniquely placed at this time and are thus represented by open bars on Fig 1

(e) The sequence previously listed by Uchida was that obtained by Fellner This oligomer has now been independently examined and the sequence is found to be as reported here (L Z and C R W, unpublished)

(f) This oligomer has been re-examined with more powerful techniques and the sequence given here is taken to be correct rather than that previously reported by Uchida

(g) These two isomers are placed here in a fashion consistent with the results of Santer and Santer[2a] However, Ehresmann suggests the opposite placement

(h) No evidence for this oligomer has been found in our preparations of E coli 16S rRNA (see Uchida) Although it is found in eight other organisms, it is not found in any Enterobactericeae or Spirillaceae 16S rRNA so far examined The oligomer is thus indicated on Fig 1 by an open bar

(i) This sequence and what seems to be the earlier version of it CAUCACG, reported by Fellner has not been found in E coli by Uchida nor has either been found in any other organism Its position is indicate(on Fig 1 as a fractional solid bar

(j) Two copies of this oligomer are reported to be very close to each othe in this area of the sequence The sequencing is, however, rather incomplet(here, and Ehresmann indicates the first copy is questionable Althougf Uchida reports 1½ copies, no other Enterobacteriaceae or Spirillaceae i: known to have two copies Thus only one copy (No 151 is omitted) i: included in Fig 1 Furthermore, the order of No 155 and No 157 is that suggested by Noller[16] rather than that of Ehresmann

(k) Fellner reports only one copy of this oligomer and Ehresmanr locates only one Uchida, however, reports 2 and every member of th(Enterobacteriaceae and Spirillaceae so far examined has been found definitely to contain 2 Thus this oligomer has been represented as ar open bar on Fig 1
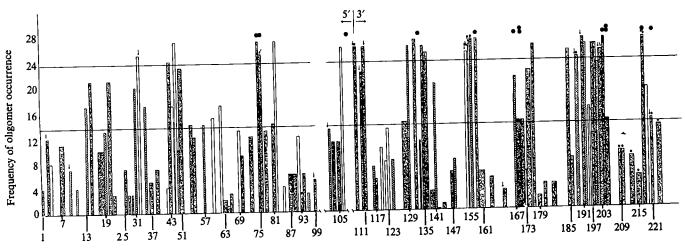
(l) This oligomer represents a minor correction to the sequences given b) Uchida (C R W, unpublished)

(m) This oligomer was previously unsequenced by both Uchida and Fellner and is now known to have the sequence indicated (C R W, unpublished)

(n) The sequence previously listed by Uchida for this oligomer was that reported by Fellner This oligomer has now been independently examinec and the sequence is found to be as reported here (C R W, unpublished)

(o) Although the 3' terminal oligonucleotide has not yet been fully sequenced in this laboratory, it is known to vary across phylogenetic lines Certainly the first five and probably the first seven nucleotides, AUCACCU are found in all but one case, and the remaining bases in all cases are a mixture of uracils and cytosines (C R W *et al*, unpub- lished)

(p) These two oligomers have not yet been located in the E coli 16S rRNA sequence by Ehresmann They are, however, reported by both Uchida and Fellner

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Relative oligomer position in 16S rRNA primary structure

Fig 1   Distribution of phylogenetic conservation in 16S rRNA Each bar represents one of the characteristic oligonucleotides of the *E coli* 16S rRNA sequence The ordinate shows the number of organisms which have been found to contain that oligomer and the abscissa indicates their exact position in the sequence of Ehresmann *et al* [4], numbers are those given in Table 1 The width of the bars is proportional to the size of the oligomers The known gaps in the sequence are indicated by (——/ /——) on the abscissa The solid bars designate unique oligomers containing six or more bases or a post-transcriptionally modified base The cross hatched bars represent unique oligomers of length five The open bars represent the most consistent placement of those oligomers which are not unique, their placement must be considered highly tentative Arrows above the bars indicate the points of kethoxal substitution, positioned so as to indicate whether the substituted guanosine is at the 5' or 3' end of the oligomer[16]. The small dots indicate known points where T_1 ribonuclease can remove an oligonucleotide from the intact 30S subunit The large dots indicate the location of post-transcriptionally modified bases that are found in *E coli* or other 16S rRNAs

to consider which of them are exposed on the 30S ribosomal subunit This problem can be approached in a number of ways (1) by determining points of nuclease attack on the 30S subunit, (2) by locating post-transcriptionally modified nucleotides (since an RNA segment must be exposed at some time during ribosome assembly to allow attack by the modifying enzyme), and (3) by determining points of attack with chemical modifying reagents All these techniques have been used, and the results are included in Fig 1

The location of post-transcriptional modifications as defined by *E coli* oligomers and those found in related sequences of the other 26 organisms correlate completely with the conserved segments Each of the six conserved regions in the 3' half of the molecule and the adjacent one in the 5' half of the molecule contains modifications in some if not all organisms No modification is found in an unconserved region of the molecule Kethoxal modifications of the intact ribosome have been made and identified[16] These correspond strongly with the highly conserved regions Nos 2, 4 and 6 which contain respectively 2, 4, 3 and 3 kethoxal attack points Attack points found by T_1 ribonuclease digestion of intact 30S subunits (T U and C R W, unpublished) also follow this general pattern This last study indicates that oligomers 209–223 are especially susceptible to attack Santer and Santer[17] found oligomers 207–217 to be susceptible in a similar study

The ribosomal protein-binding sites so far characterised seem by and large not to coincide with the conserved regions[18-21] All the primary binding proteins (with perhaps one exception) can be localised in the 5' half of the molecule There does exist, however, a ribonucleoprotein subparticle containing proteins S7, S9, S13 and (usually) S14 (ref 22), that includes some oligonucleotides from region No 2 (its 5' portion) This in itself is not proof that region No 2 actually contains a protein-binding site

If this apparent negative correlation proves entirely correct it raises an interesting problem Since a functional 30S ribosome chimaera can be constructed from *E coli* 30S proteins and *B stearothermophilus* 16S rRNA[23], one would assume homology of the protein-binding sites in the two organisms The apparent contradiction would be resolved if homology were in this instance to be primarily in secondary or tertiary structure and so on There is a precedent for such an interpretation in the 5S rRNA This molecule is functionally interchangeable between the

above two organisms[24], yet it is likely that its ribosomal protein-binding sites are again not in the regions of highest primary structural conservation[25,26]

The results reported here show the extent to which each of the characteristic ribonuclease T_1 oligomers of *E coli* 16S rRNA is conserved The universal and highly conserved oligomers cluster, falling into not more than nine regions These groupings are not uniformly distributed throughout the molecule, but rather are concentrated in the 3' half where they alternate regularly with regions of low conservation At least seven of the regions are located on the surface of the 30S particle (at some stage), including all of those in the 3' half of the molecule These results strongly suggest that important functional features of the 16S rRNA are located in the 3' half of the molecule Since little or no correlation exists between these conserved regions and known ribosomal protein binding sites, the implication is strong that large areas of the RNA are directly involved in ribosomal function

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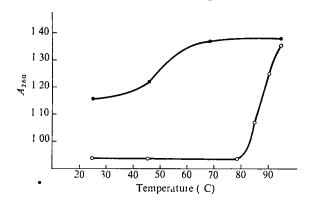
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absorption spectrum was obtained with a maximum at 260 n1 the ratio of A_{260} to A_{235} was 2 28 The temperature-abso bancy curve for MgDNA, dissolved in deionised water, shown in Fig 1, T_m for MgDNA was 85–87 °C, the return c cooling was about 50%, and the hyperchromicity was 43° This contrasts with the T_m for spleen NaDNA in deionise water, which was 71 °C In both cases, the initial absorban at 260 nm was closer to 0 95 The melting profile for MgDN was steeper than that for NaDNA, indicating extensive stabi sation of the DNA double helix by Mg(II) The ratio of Mg(I to DNA(P) was 0 518, indicating one magnesium per tw nucleotide phosphates MgDNA was also stable to hydrolys by pancreatic DNase I in the absence of added Mg(II) additic of Mg(II) to the above solution resulted in rapid degradatic of the DNA[5] DNase I requires free Mg(II) for activity, thi very little of the Mg(II) in MgDNA was available

Solutions of single-stranded NaDNA were prepared t heating to 95 °C and cooling quickly, solutions of nativ bovine NaDNA in deionised water This process was repeate until the melting curve of the resulting solution showed n rise in absorbancy when heated

Formation of triple-stranded bovine DNA *in vitro*

EVIDENCE for a triple-stranded complex of polynucleotides was reported for homopolymer RNAs by Felsenfeld *et al*[1] in 1957 They detected a drop in absorbancy at 260 nm for a mixture consisting of two parts polyadenylic acid (poly(rA)) and one part polyuridylic acid (poly(rU)), during a continuous variation mixing study of the two homopolymers Addition of 0 1 M $MgCl_2$ produced the greatest triple-strand formation with a minimum absorbance at 67 mol % poly(rU), 33 mol % poly(rA)[2] Since this early work, many triple-stranded complexes have been identified, including combinations of homopolymers of deoxyribonucleotides and ribonucleotides, Riley *et al*[3] reported a triple strand formed by the homopolymers dA dT. dT They also reported the caesium sulphate buoyant densities of various double-stranded and triple-stranded homopolymers In all cases, the triple-stranded homopolymer had a greater buoyant density than the analogous double-stranded homopolymer We now report evidence for the formation of triple-stranded bovine DNA *in vitro*

We used a magnesium salt of bovine DNA, which was prepared from bovine spleen by substituting $MgCl_2$ for the sodium chloride–sodium citrate medium (SSC) in a standard DNA preparation procedure[4] Sodium xylene sulphonate (Naxonate G) was added to precipitate protein The MgDNA so prepared was purified by reprecipitating with cold 2-propanol three times, and the final precipitate was dried by washing first with ethanol and then with acetone When the purified MgDNA was dissolved in 5×10^{-4} M $MgCl_2$, the usual DNA

Fig. 1 Temperature-absorbancy curves for MgDNA in deionised water Absorbance was measured at 260 nm ○, Heating curve, ●, cooling curve

Fig 2 Continuous variation mixing curves for mixtures of native bovine MgDNA and denatured NaDNA The unbroken line is the expected curve for no interation, % denatured NaDNA is mol %, and the absorbance was measured at 260 nm *a*, MgDNA and NaDNA in 0 1 M $MgCl_2$, *b*, MgDNA and NaDNA in deionised water

Continuous variation mixing curves, as used by Riley[3] and others, were made to determine the interaction of MgDNA and denatured NaDNA Figure 2 shows mixing curves for the two DNAs (*a*) in the presence of 0 1 M $MgCl_2$ and (*b*) in water only The unbroken line is the expected curve for no interaction (0 1 M $MgCl_2$ was found to be the optimum concentration of $MgCl_2$ in our studies, and for homopolymers, by Rich[6]) Figure 2 clearly shows a one-to-one interaction between double-stranded MgDNA and single-stranded Na-DNA, in the presence of Mg(II) ion These experiments were

Table 1 Summary of data from continuous variation mixing curves for double-stranded bovine DNA with single-stranded NaDNA

Type of DNA Double strand + single strand	Added salt	mol % SS NaDNA at A_{260} minimum	DNA ratio at minimum
MgDNA + NaDNA	None	No min	—
MgDNA + NaDNA	0 1 M MgCl$_2$	50%	1 1
NaDNA + NaDNA	SSC	No min	—
NaDNA + NaDNA	0 1 M MgCl$_2$	No min	—
MgDNA + Salmon sperm NaDNA	0 1 M MgCl$_2$	No min	—

repeated with double-stranded NaDNA plus single-stranded NaDNA in the presence of SSC as well as 0 1 M MgCl$_2$ The experimental results are summarised in Table 1, no interaction was apparent in these mixtures That the reaction may be species specific is indicated in the last experiment reported in Table 1, where denatured salmon sperm NaDNA was substituted for denatured bovine DNA, and no drop in absorbance was observed

The time required for the formation of the triple-stranded complex was less than 30 s at room temperature Felsenfeld[2] found that triple-strand formation took about 24 s with the homopolymers rA and rU in 0 12 M MgCl$_2$

The amount of triple-stranded bovine DNA formed was estimated by the drop in absorbancy at 260 nm (Fig 2) as compared with the values reported for homopolymers If we assume that the homopolymers formed a 100% triple-stranded complex, then our DNA was between 15% and 20% triple-stranded We attempted to separate the various species of DNA (single-stranded NaDNA, double-stranded MgDNA, and triple-stranded) present in the one to one denatured NaDNA and native MgDNA mixture in 0 1 M MgCl$_2$, by sucrose density gradient centrifugation and by CsCl density gradient centrifugation In all such attempts, we found only one peak caused by DNA, which indicated one species of DNA in the solution The CsCl$_2$ buoyant density of this DNA was

no further increase in absorbance at 260 nm was observed The minimum time required was 4 h The digest was then separated by gel filtration through a column of Sephadex G-200, 40×1 cm, pretreated with 0 10 M MgCl$_2$, which was also used as the eluant Unhydrolysed DNA was eluted immediately after the void volume and accounted for 18% of the applied A_{260} units The balance of the 260 nm absorbing material appeared in a complex peak about ten 2 5-ml fractions later, which corresponded well to the elution volume of a mixture of known deoxyribonucleotides The fractions containing the DNA were pooled, and then concentrated by addition of dry Sephadex G-200 After swelling and water regain had occurred, the mixture was filtered with suction This final filtrate contained DNA which was resistant to DNase I digestion, that is triple-stranded DNA, and was used to determine the CsCl buoyant density of the pure triplex Triple-stranded DNA had a buoyant density of 1 726 g cm^{-3} compared with double-stranded NaDNA and MgDNA (both were 1 699 g cm^{-3}) and agrees with the values reported for triple-stranded homopolymers[3,7,8]

The temperature–absorbancy curve for the pure triple-stranded DNA fragments in 0 1 M MgCl$_2$ is shown in Fig 3 The T_m was 79 °C and the hyperchromicity was 33% The T_m and hyperchromicities of our three DNAs are compared in Table 2 Since the melting curve of the triplex produced a single break, the three strands seem to be bound equally and are quite stable in 0 1 M MgCl$_2$

Table 2 Comparison of melting temperatures (T_m) and hyperchromicities of single-stranded, double-stranded and triple-stranded DNA

DNA	T_m (°C)	Hyperchromicity (%)
Denatured NaDNA*	65	13 5
Native MgDNA†	86	43
Triple-stranded DNA*	79	33

* In 0 1 M MgCl$_2$
† In deionised water

The biological significance of triple-stranded DNA can only be speculated upon, but it is valuable as a model of possible DNA RNA triple-strand formation during transcription Zubay[9] postulated such a triple strand and concluded that its formation required a widening of the major groove of the DNA A short triple-stranded DNA segment has also been proposed as an intermediate in DNA replication[10,11] The fact that MgDNA was required for triple-strand formation may be explained by the effect of Mg(II) on the DNA helical structure, in contrast to Na$^+$, Mg(II) would be expected to bring the phosphate moieties of the opposing strands closer together, thus the major groove of the DNA helix would be widened and triple-strand formation would be possible

Fig 3 Temperature–absorbancy curve for pure triple-stranded bovine DNA in 0 1 M MgCl$_2$

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1 681 g cm^{-3}, when compared with the bouyant density of native DNA (1 699 g cm^{-3}) A single species of DNA, resulting from the above mixture, can be visualised as a core of double-stranded MgDNA, to which is attached single-stranded DNA to form short segments of triple-stranded DNA, with many dangling tails of single-stranded NaDNA, and many areas of MgDNA that are solely double-stranded

To determine the true buoyant density and melting curve of pure triple-stranded DNA, it was necessary to remove all single-stranded and double-stranded segments from the crude single triplex This was done by exhaustive digestion of the triplex mixture with pancreatic DNase 1 (2 µg ml^{-1} at pH 6 8) until

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The phosphorylated site of calf thymus F2b histone by the cyclic AMP-dependent protein kinase

THE phosphorylation of histones is a complex process which occurs at different stages of the cell cycle[1-3] Different types of histone kinases are responsible for the phosphorylation of the seryl (or threonyl) residues of the histone fractions One of them is the ubiquitous cyclic AMP-dependent protein kinase[4,5], whereas the histone kinase predominant in mitotic cells is not activated by the cyclic nucleotide[6] The cyclic AMP-dependent enzyme of rat liver, phosphorylates the seryl residue in the position 37 of calf thymus F1 histone, and the growth associated histone kinase catalyses the phosphorylation of other seryl and threonyl residues of the F1 fraction[4-6]

The cytosol and nucleus of human tonsillar lymphocytes also contain cyclic AMP-dependent and independent histone phosphorylating enzymes[7,8] In vivo, F2b histone is phosphorylated to a small extent only[1-3] but in vitro it is about as good a substrate as F1 for both of the lymphocyte histone kinases, and a better substrate than the other histone fractions The lymphocyte enzymes, however, differ from each other in their kinetic parameters and in the

sites of phosphorylation of the F2b fraction[7,8] When pho phorylation was carried out using the cyclic AMP-indeper dent enzyme four different, approximately equally phosphorylated peptide fragments were detected on th two dimensional paper electrophoretic pattern from th tryptic digest of F2b histone fraction, whereas one of ther was phosphorylated preferentially when the reaction wε catalysed using the cyclic AMP-dependent enzyme[7,8] Th same single peptide fragment of calf thymus F2b wε phosphorylated also by the cyclic AMP-dependent enzym of rat Harderian gland[9], hence, this specificity seems to b the common feature of cyclic AMP-dependent protei kinases of different species and of different tissues

The amino acid composition of the tryptic peptide fraξ ment phosphorylated by the cyclic AMP-dependent enzym has been determined (Table 1) The complete amino aci sequence of the F2b histone fraction is known[10], and th amino acid composition of the phosphorylated peptide i consistent with that of only one tryptic fragment of F2t There is a single tryptic peptide containing glutamic acid serine, trypsine, valine and lysine together, that is, th residues from Glu-35 to Lys-43 (Fig 1) In addition, th C-terminal sequence of the isolated peptide, (Val-Tyr-Lys COOH, see legend to Table 1) agrees well with that of th F2b fragment in question

The phosphorylated segment of F2b contains two sery

Fig. 1 Amino acid sequence of calf thymus F2B histone[10]

Table 1 Amino acid composition of the phosphorylated peptide fragment of calf thymus F2b histone

Amino acid	Concentration (nmol)	Molar ratio to lysine
Serine	280	2
Glutamic acid	160	1
Valine	280	2
Tyrosine	220	>1, 5*
Lysine	150	1

F2B was purified according to the method of Oliver et al[12] and phosphorylated using the cyclic AMP-dependent protein kinase of tonsillar lymphocytes The incubation mixture contained 10^{-6} M cyclic AMP and 1 25×10^{-4} M γ ^{32}P-ATP (16 mCi mmol^{-1}) Generally one out of every five molecules of histone was phosphorylated in these conditions The incubation procedure, the precipitation and tryptic digestion of the phosphorylated samples were performed as described previously[4,5] The ^{32}P-labelled phosphopeptide was isolated as before[4,5] applying two-dimensional paper electrophoresis at pH 6 5 and at pH 1 9 The labelled peptide fraction was further purified by ascending paper chromatography (pyridine isoamyl alcohol water—35 35 30 by volume) A sample of the purified phosphopeptide was hydrolysed in boiling HCl (5 7 N) for 24 h at 105°C, and the amino acid analysis was performed on a JEOL JAH 6 analyser The C-terminal sequence of the isolated peptide was determined according to the method of Sajgó and Dévenyi[13] A sample of the peptide (40 nmol) dissolved in 0 1% NH$_4$ HCO$_3$ was digested by carboxipeptidase A and B (SERVA, free of trypsine and chymotrypsine) at 37°C The molar ratio of enzyme to substrate was 1 100 Aliquots were taken out after 0, 5, 15, 30 and 60 min digestion into 0 01 M HCl The aliquots were freeze-dried then resolved in 0 01 M HCl and were put on a resin-coated chromatoplate (FIXION 50-X8, Chinoin, Hungary) Control mixture of all the amino acids in 0 01 M HCl was also put on the chromatoplate After ascending chromatography in sodium citrate buffer (Na$^+$ 0 4 M, citric acid 0 4 M, pH 3 28) at 45°C, the amino acid spots were developed using ninhydrin–cadmium–acetate–collidine reagent After 5 min digestion the only detectable amino acid was lysine After 15 minutes digestion a small amount of tyrosine appeared and the amount of lysine increased In the last two samples a spot in the position of valine could be observed, beside the strong tyrosine spot
*This method destroys a part of the tyrosine residues

groups in positions 36 and 38 Our data provide no information as to which one is modified but the electrophoretic pattern suggests that only one of them is phosphorylated Assuming phosphorylation of Ser-36, thus Arg-34-Lys-35 of F1 and Arg-33-Lys-34 of F2b are identical residues in the vicinity of the sites phosphorylated by the cyclic AMP-dependent kinase, although the surrounding sequences differ significantly in calf thymus F1 (ref 11) and F2b histone fractions Considering the total molecules, however, the similarity between the positions of the phosphorylated groups is obvious and the modification occurs adjacent to the N-terminal clusters of basic amino acids in both cases

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reviews

ACCORDING to the foreword, the course on which this book is based aimed at providing both a comprehensive survey of basic topics and a review of some more specialised topics of great current interest and intrinsic importance On that criterion it fails For example, I find it hard to find the great current interest or intrinsic importance in a three page contribution on rocket soundings in Poland But then, I'm not Polish

Had this book been the proceedings of a conference of atmospheric specialists then its contents could not have been faulted, but on the basis of the stated aim I have a vision of eager young workers, new to aeronomy, lusting for understandable and interesting surveys What they will receive is a set of 16 uncoordinated and rather specialised lectures that present an unbalanced view of the upper atmosphere

I graded the articles for interest and clarity by pretending that I was a first year graduate student The results

α Tides (Lindzen), Electrical Structure (Webb), Rockets and Remote Sensing (Heath *et al*), Lower Ionosphere Morphology (Harnismacher), Winds and Turbulence (Muller), The F Region (Rishbeth)

β Photochemical Models (Hesstvedt), D Region Measuring Techniques (Rumi), Composition Studies (Van Zahn), Corpuscular Effects (Mariani)

γ The other six articles

The γs account for only 50 pages, but their omission would have im-

Air text

Tom Beer

Structure and Dynamics of the Upper Atmosphere (Proceedings of the Second Course of the International School of Atmospheric Physics) (Developments in Atmospheric Science, 1) Edited by Franco Verniani Pp xiii+535 (Elsevier Scientific Amsterdam, Oxford and New York, 1974) Dfl 160, $61 50

proved the book They are low quality research contributions that should have been sent to the appropriate research journals It is a mockery to have a four page article grandiosely entitled "Mid-Latitude Sporadic E" (Bossolasco and Elena), it fails to do credit to an exceedingly important subject

Had I graded the contributions in terms of their completeness then almost all of them would have been relegated to the βs and γs We are presented with 40 pages on winds and turbulence in the meteor zone without the merest whisper of sporadic E (E_s) Harnismacher, to his credit, includes E_s morphology in his excellent contribution but it would come as a surprise to Verniani's scholars at Erice that theories actually exist to account for E_s They would have left the school, after 535 pages of heavy monologue, unaware of spread F, polar cap absorption, the plasmapause, the polar wind, incoherent scatter radars, the acoustic

cutoff frequency, aurorae and the details of the dynamo current

Overall, I think the editing was lamentable The greatest omission was of an introductory chapter that defined a few things Like upper atmosphere, ionosphere, stratosphere, D region, and so on Also, the book is a very uneven mix between theory and experiment Some topics (such as tides) are only dealt with theoretically, and only the experimental details of others (for example, lower ionosphere) are presented To complete the confusion, half of the articles use c g s units whereas the other half use m k s

On the credit side, the editor has carefully systematised everybody's bibliographies, the author index and subject index are well prepared, there are surprisingly few typographical errors, and Elsevier have done a fine job on the printing and binding I unintentionally dropped the book from the back of my speeding motor scooter, an event which the binding has uncomplainingly accepted

I would only recommend this book to those already actively engaged in work on the upper atmosphere A few of the articles are excellent, though the active worker will have seen much of the material before in one form or another Aeronomy desperately needs a lot less edited collections of unrelated papers and a few more single or co-authored works of simple exposition and of authority I hope that *Developments in Atmospheric Science, 2* provides the latter □

THIS book would make a good present for the serious aquarist and would be a useful addition to any departmental library which gives a thought to fostering an interest in freshwater fish and their care It is not a new book, but a new edition of an old one An earlier issue was reviewed by Phillip Greenwood in these pages (*Nature*, **198**, 516, 1962) and he found much good in it I concur with his view

The new edition is compact, strongly bound, and presented as two volumes The bulk of the text comprises descriptions of the species grouped by families Each description contains general information at the family level, with distribution charts, and the accounts of the species include more detailed material, biometric data, and illustrations For the aquarist's special needs information as to behaviour, food

preferences and environmental requirements with special reference to breeding, are also given

Some changes have been made in the

Fish out of date

F. R. Harden Jones

Freshwater Fishes of the World By Gunther Sterba Vol 1 pp 1–456, vol 2 pp 457–877, 191 plates (Studio Vista London, 1974) £12 50

new edition Illustrations that were coloured in previous editions are here in black and white, and a whole set of new colour photographs have been included they are good The page size has been somewhat reduced and although this makes the book more compact, there is the disadvantage that

drawings originally scaled to natural size are now 10% too small

And what of the book's deficiencies? These must be measured against one's expectations, I would set those out as 'identification', 'classification', and 'description' The book fails to clear only the first of these hurdles in that there is no key reference to families, for as Denys Tucker—translator and reviser—notes, that section was stillborn A pity, because it would have put the finishing touches to what is a useful work There are some trivial mistakes —what book is free from these?—and I would be disappointed to learn that they had been carried through from earlier editions And no changes have been made to the select bibliography, in which the most recent publication is dated 1962, for £12 50 a purchaser could expect to be taken into the 1970s

Allelopathic response

Allelopathy (Physiological Ecology A Series of Monographs, Texts and Treatises) By Elroy L Rice Pp x+ 353 (Academic London and New York, September 1974) $25 00, £12 00

MANY people are familiar with the interactions between microorganisms which involve the release of chemical toxins, yet the general public and even many biologists are probably unaware of the importance of such toxic sub-stances in interactions between higher plants But the concept is by no means new De Candolle was perhaps the first biologist to suggest such a possibility in 1832 Yet, in spite of that, this book can justifiably claim to be the first volume in English to be devoted to the subject That alone serves to illustrate the neglect which the study of allelo-pathy has suffered, it may also be re-garded as an indication of the difficulties and uncertainties which surround such a study

The definition of allelopathy used in the book is that of Molisch, which in-cludes chemical interactions between plant and plant and between micro-organism and plant The study of chemical interactions between plants and animals (included in the term 'allelochemics' as used by R H Whit-tacker) is thus excluded The emphasis of the book is ecological, and the role of allelopathy in the development of community structure and composition forms a theme linking several sections, whether dealing with phytoplankton, weeds in an old field succession, fire cycles or vegetation pattern in stable communities

Two chapters on the role of allelo-pathy in agriculture and horticulture deal briefly but effectively with the considerable volume of work which has been done on this subject A further section is devoted to the chemistry, physiology and mode of action of the inhibitors involved in allelopathy The final chapter on chemical reactions be-tween animals and plants is rather in-congruous It is too brief to do justice to a fascinating aspect of plant toxicity and since it does not fall within the definition of allelopathy used by the author, it hardly seems necessary

Overall, however, the coverage is good and the bibliography excellent In emphasis and treatment this is essen-tially a personal view of the subject by Elroy Price He is well known for his work and ideas on the inhibition of bacterial processes—such as nitrifica-tion—during the development of stable vegetation, and on the role of allelo-pathy in delaying succession in some instances by the attainment of domin-ance by species with inhibitory proper-ties These subjects and others like them are developed fully in this book

At times readability and flow ar sacrificed for the author's concern t give practical details (even to the grad of Whatman filter paper used in ex periments), but summary paragraphs a the end of chapters make up for this

I cannot agree with the author's con cern to divorce the concept of allelc pathy from that of competition Th plant which has evolved allelopathi properties is comparable with a specie of coarse or robust morphology in tha it is able to substitute space for resource in competitive interactions On acquiring such space the resourc may become freely available to th plant which would not necessarily re quire any degree of efficiency in tap ping it To say that the struggle fo space is not competition is, in m opinion, an exercise in semantics

This book is a full and stimulating account of a sadly neglected subject, a such it should be read by all practisir ecologists, physiologists, agriculturalis and foresters It is to be hoped that r production will give impetus to furthe research in an area where many prol lems, both theoretical and practical have yet to be solved **Peter D. M**

Nuclear spectroscopy

Nuclear Spectroscopy and Reactions vols 40-A and 40-B Edited by Joseph Cerny Part A, pp xvii+518, part B, pp xvii+711 (Academic New York and London, October 1974) Part A $44 50, £21 35 Part B $49 50, £23 75

THE rate of growth of the physical sciences is nowadays so great that ever the specialist has difficulty in keeping abreast of more than a very narrow field Yet he needs to know more than this, so it is very important tha specialists should periodically summaris the work in their field for the benefit of those working in related areas

Professor Cerny has succeeded admir-ably in bringing together the work of a group of well known specialists in nuclear spectroscopy and reactions, and has arranged it in four volumes

The authors of the articles in the first two volumes—covering nuclear instru-mentation and research in nuclear spectroscopy—are among the foremost authorities on their subjects and in many cases are leading the most active groups in the field The result is most impressive and contains within a rela-tively manageable compass expert and up-to-date coverage of many of the important growth areas in nuclear spectroscopy The volumes will be essential reading for all engaged on work in this field The subsequent volumes, on gamma-ray spectroscopy and theoretical analysis, will be eagerly awaited   **P. E. Hodgson**

nature

The unloved treaty

IN two months time those countries which have ratified the Non-Proliferation Treaty (NPT) will be reviewing, under UN auspices in Geneva, the first five years of the treaty There is no doubt that in 1970 the hopes (at least of the non-nuclear countries) were that by 1975 the NPT would be a solid foundation on which to build further arms-control measures, aimed at progessively steering the world away from the contemplation of nuclear solutions to political problems Certainly in the past five years nobody has fired a nuclear weapon in anger, though the NPT could hardly claim much credit for that On the other hand the prospects for a nuclear-free future are no better than they were in 1970 and the NPT foundations have been neglected—indeed show signs of crumbling

The treaty bans the development of nuclear explosives of any kind in non-weapon states in exchange for promises that the benefits of peaceful applications will be extended to those states There are some notable absences from the list of ratifiers, a few non-ratifiers have signed but will not ratify for fairly specific reasons, but the following countries *inter alia* have not even declared interest in the treaty at all   Argentina, Brazil, Chile, China, France, India, Israel, Pakistan, Portugal, South Africa and Spain The weight of the three original nuclear powers which used to reassure other countries that treaties were worth signing is now counterbalanced by the weight of three other nuclear powers who want nothing to do with this treaty

The reasons for the failure of the NPT to catch on are all too obvious—it is asking for major concessions from the 'have-nots' in exchange for minimal concessions from the 'haves', and it is a trap for have-nots who sign and find that their unfriendly neighbours do not follow suit Much has been written on the extent to which vertical proliferation, the continued refinement of the nuclear nations' arsenal, causes offence to non-nuclear powers There has been remarkably little public discussion of the real reason for the diffidence of most potentially nuclear states towards the NPT—the fear of unbalanced horizontal proliferation which, in the absence of simultaneous accession by all states, cannot be ruled out

It is widely believed that the NPT contains assurances to non-nuclear states in the event of nuclear attacks This is not so A resolution of the UN Security Council, which appeared when the NPT was first opened for signature, replaces any binding commitment by a general welcome for the intention of the UK, the USA and the Soviet Union to act "in accordance with the Charter" were any non-nuclear-weapon state, party to the NPT, to be the victim of an act, or the object of a threat, of nuclear aggression The catch is that the Security Council with all its possibility for veto would be the vehicle for action Small wonder that countries which have refused to sign the NPT abstained on this resolution also

Finally, the NPT promised that benefits from peaceful nuclear explosives would be made available "through an international body" Negotiations were to start "as soon as possible" Back in 1968 when the treaty was drafted, the Plowshare programme in the United States was languishing In major excavation projects, for instance, costs for even chemical-explosive techniques were double those for conventional techniques Since then, however, things have changed dramatically The factor of two in cost is now a factor of much less than one, in the future the same will be true of nuclear explosives

In the Soviet Union, in an entirely different political and physical environment, the cross-over point must have been reached much earlier, for nuclear explosive projects burgeoned in 1970 and now run at the rate of 10 a year Thus the hopes of many in 1968 that peaceful applications would never be economic and thus might not obstruct arms control discussions have been proved unfounded

So where is the international body? It is now five years since the treaty came into force and the International Atomic Energy Agency (IAEA) has yet to establish any body to consider the political and legal aspects of an international service In the meantime, the Soviet Union, it is said, took it upon herself to offer India nuclear explosives for peaceful purposes but was rebuffed Some minds had better be made up soon on the establishment of a body, the next bilateral deal may be accepted

The IAEA has not been entirely inactive Technical panels have exchanged a substantial amount of information and non-nuclear countries have been party to all this But the political nettle has yet to be grasped

The reasons are a combination of bureaucracy, politics and genuine concern for safety There is a resistance in Vienna to the accumulation of yet more duties for a secretariat already fully extended There is concern that the IAEA might include only NPT-ratifiers amongst beneficiaries of nuclear technology And there is a fear that the apparent legitimisation of nuclear explosive transfers will lead to the even wider dissemination of potential weapons material, and will be an encouragement to even more countries to think nuclear This last is a fairly weak objection now that events have overtaken hopes that peaceful explosions might not prove viable The IAEA itself these days puts out fairly enthusiastic publicity for nuclear explosives in its technical reports There is, however, some substance to fears that the service might be tied to the NPT Any restrictions of this sort, although at first sight a means of bolstering up the treaty, would in the long run diminish IAEA initiatives by driving non-ratifiers into more implacable opposition

On many grounds the NPT has not lived up to expectations and could even be seen as creating opposing nuclear camps Will anyone have the courage in May to suggest the treaty be scrapped and new approaches tried?

AS a country Britain is living beyond its means, and it is surely no more than good sense to consider ways in which we can meet more of our needs from our own resources, among which agricultural resources figure prominently In the past 100 years or so the latter have been evaluated against a background of abundant world supplies of food, available freely except for short periods of war and post-war restriction, and to be purchased cheaply in relation to the price of the industrial goods we had to offer in exchange We have rarely had an explicitly formulated agricultural policy, but the *de facto* policy that has grown up has resulted from the impact of these world forces

The world situation has changed, dramatically and to our disadvantage Although agricultural production has increased enormously over the past quarter century, the increase has been taken up by population growth and by the increasing sophistication of the diets of affluent countries Thus, now that the basic resources on which farm production depends—land, fertilisers and energy—have become scarce and costly, we have no margin to protect us against shortage, prices have risen greatly in rich countries, and poor countries face widespread hunger In this context we should reappraise our agricultural assets, with a view to meeting from them more of our needs

In Britain we enjoy a generous, not to say extravagant, diet, including a high proportion of animal products The livestock industry has come to depend very heavily on concentrate feeding, and home feed production is augmented by massive imports of coarse grains and protein feedstuffs In present circumstances this is a profligate way of feeding ourselves, and we know that we could devise a palatable and nutritionally adequate diet at much less expense in real resources, and with much less dependence on imports A reduction in the heavy consumption of animal products would make a major contribution to Britain's balance of payments, and would help ease the world food situation

The corresponding changes in the production pattern of British agriculture would require extensive planning, and would take some years to implement in full It is, therefore, particularly important that we should start planning without delay Planning of this order is beyond the time scale of political parties and exceeds the sectional interests of farming organisations But it is vital if agriculture is to contribute more substantially to the solution of our economic problems Some of the major topics to be considered in such an enterprise are out-

Agriculture and nutrition

Almost overnight the scientific community at large has discov of much more attention. Sir Joseph Hutchinson argues for a : (left). Professor Jean Tremolières points to the need to stimula

lined here, in the hope of encouraging interested people to get together, to study agricultural policy in depth, and to make known their conclusions

● **Cropping pattern.** Cereals are the basic foodstuffs of the human race In the British diet we consume approximately 90 kg a head a year directly, and use about 360 kg a head to feed to livestock which produce meat, milk and eggs For this we grow 13–15 million tonnes and import 8–9 million tonnes How would one implement a decision to (a) halve, or (b) eliminate cereal imports? This would fall under two heads, first, the replacement of imported cereals used in human food, chiefly by growing good milling instead of low quality winter wheats, and replacing some of the spring barley crop with good quality spring wheats, and, second, the reduction of cereal-based livestock production to match the reduction in the availability of feed grain Of this we have the experience of the Second World War, when pig and poultry stocks were drastically cut back, and beef production was limited to the available grass feeding acreage

Other crops would not be so directly affected Potatoes would probably remain about as at present There might be considerable debate about sugar beet, but considering the need of the Third World for access to industrial markets, and the disfavour with which sugar is regarded by nutritionists, it seems likely that the crop should be stabilised at a level appropriate to use as a rotation crop in arable farming

In a diet with a lower meat content, vegetables might have a larger place, and vegetable crops might be increased Oilseeds are almost entirely imported, and oilseed crops should be reviewed Ley breaks in arable rotations would assume greater importance, and are considered in relation to the maintenance of fertility

● **Fertility levels** The present high fertility of British farm land has been achieved by the liberal, and at times almost profligate, use of fertilisers Now that these have become expensive and scarce, there is no doubt that considerable savings can be made by more discriminating fertiliser practice Greater savings are to be gained by better distribution of animal manure,

and by the use of leguminous crops : augment the nitrogen supply The: two are interrelated, since the tenden(to separate crop production froi animal industry has resulted in an u convenient concentration of anim; manure on livestock farms and an abai donment of grass/legume leys on arab. farms

The redistribution of crops and stoc in order to maximise the conservatic of fertility would be the most importai and the most difficult consequence (this kind of reappraisal of British agr culture The rundown of intensive liv stock enterprises would involve writii off capital in intensive housing, ai the reintroduction of grazing livesto: on intensive arable farms would requr investment in fencing, water supl and some winter housing How much At what rate? And where?

● **Human nutrition.** Human nutri'io lags behind animal nutrition on accour of the difficulties of experimentatio with human subjects The diversity c opinion among eminent nutritionists : to the causes of the 'diseases of affli ence' is sufficient indication of th uncertainties of the subject Neverth less, the existence of diseases associate with affluence is not in dispute, an this, together with economic need an the limitations of world food suppl; is a powerful reason for devising die that are less demanding on resource: and perhaps less menacing to health

In conclusion, these are three majc areas in which we have substanti; room to manoeuvre If we are t exploit the freedom of choice open t us with a view to realising the fulle: potential of our agricultural resource some of us must get down to the har work of studying these topics, an charting a course of action in detai Some groups are already concerned 1 this area, notably the Joint Consul ative Organisation recently set up b the Ministry of Agriculture, Fisherie and Food to give guidance on researc priorities, but we need a wider asses: ment of the place of British agricultur in British economic policy Are w interested to provide a factual basis o which informed opinion can be buil or are we content to stumble from Pric Review to Common Agricultural Polic negotiation without real understandin of where we are going? ⊏

ashionable subjects are worthy
n of British agricultural policy
al research (right).

THROUGHOUT the crises of our time—the financial crisis, the troubles of society, the energy crisis—our idol, science, has remained unquestioned. Now the time has come to ask ourselves what science can contribute.

This is what has just been done in the field of human nutrition by a British committeee convened jointly by the Medical and Agricultural Research Councils (MRC and ARC) under the chairmanship of Professor Neuberger. Its report is the collective testimony of a number of scientists with long and converging experience. Until now nutrition has appeared to be limited to certain specialised functions, to the study of deficiency diseases and to dogmatic ideas on diet. In fact, nutrition is not just a particular function of living beings; together with immunology and genetics, it is one of three universal concepts which relate human beings to their environment and to the society in which they live. There was a need to restore nutrition to its true dimension as a synthesis of sciences, a culture which stimulates and fertilises, and to get away from an approach which had become too purely analytical.

Why should it be surprising that it is the bread man eats which serves as the starting point for taking a new look at our attitude to science?

The disorganised state of our knowledge of nutritional science could not last. Nutritional requirements as laid down by the expert committees are a mass of contradictions, arising from a scarcity of facts which in any case have little meaning. For example, energy requirements represent the actual consumption of the reference man; yet this Apollo is a man of our industrial society whom we know to be too fat and who eats too much. On the other hand, protein requirement has a physiological basis, the minimum values having been derived from measurements made in the laboratory during brief experimental balance periods. No society actually lives at this level, however, since if energy requirements are met, even the simplest diet would provide surplus protein according to these standards. The values recommended for vitamins are saturation values which have no clear physiological meaning. Hypovitaminosis is rarely seen now, but nevertheless people are trying to use these recommended values

as a basis for labelling and advertising of dietetic products in the USA and in France. Supplementation with amino acids or iron has mostly been based on economic considerations.

In the field of diseases associated with nutrition, the inability of classical physiology to explain obesity is obvious. Measurements made over 10 minutes are extrapolated to 24 hours. The physiologist applies the law of surface area which is only valid to $\pm 30\%$; yet a systematic error of 10% would represent a weight change of 13 kg a year. The cause of degenerative disease of the heart and blood vessels has been attributed variously to the percentage of calories from fat, the ratio of EFA to NEFA, to sugar and to cholesterol. Yet this is a global state, a multifactorial syndrome, in which the causal factors are interdependent.

It is the ideas expressed in the Neuberger Report, the lines of thought, the strategy, which are important, not the details. The sergeant-major will always find something wrong with the buttons: there are no references, and some important points about dietary behaviour have been neglected, but the positive side of the report is such a relief and offers such hope that one can overlook the omissions. Doctors, biochemists, physiologists, agronomists, toxicologists, dietitians, the food industry and sociologists have all co-operated and succeeded in producing a unification of knowledge which is a truly significant synthesis.

This group of academics has emphasised the 'social' function of research in relation to the health of the individual and of the community and has tried to apply this research to a number of social and medical problems. To those of us who have lived for the last 10 years with the research policy of the Institut Nationale de la Santé et de la Recherche Médicale and of the Conseil National de Recherches Scientifiques, this report under the signature of Professor Neuberger is a bomb which will breach the ramparts.

The evidence given here shows nutrition as a special area of interaction between man and his environment. The problems of toxicity and pollution are looked at from the biochemical and physiopathological point of view and the metabolism of foreign substances has been dealt with in the same way as that of nutrients.

It is, however, at the molecular and cellular level of organisation that the most practical problems of medicine, of toxicology, of *l'alimentation* have to be solved. Again it must be realised that it is the synthesis, the common language, the ideas, which will allow specialists in each sector of this huge spectrum to understand one another clearly in order to produce the illu-

mination of which man and his society are in need.
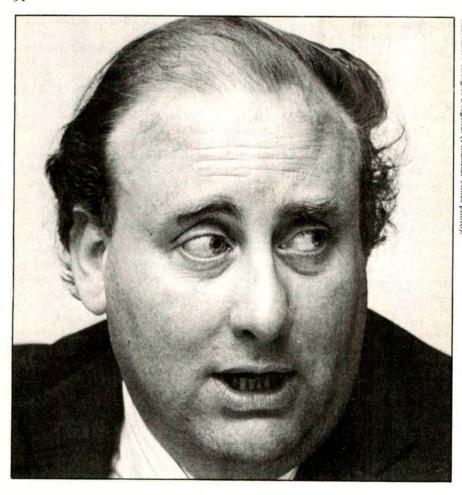
Some of the principal nutritional problems have been mentioned as illustrations. It is not possible to summarise what is already a summary of 180 pages. This kind of committee does not usually adopt an attitude so widely based on biology, so critical and so positive. There are certainly many points for discussion, but there are so many positive suggestions that all nutritionists will be obliged to meditate on them. One is more stimulated by this report than by a classical scientific publication. It seems to me very possible and desirable that different national committees should take this document, study it in depth, fill the gaps and propose different approaches to certain points.

It is appropriate here to mention that the French effort, small though it is, operating through the Fondation Française de Nutrition and the Committee on Human Nutrition of the Centre National de Coordination des Etudes et Recherches sur la Nutrition et l'Alimentation, has embarked without previous consultation on a programme which, in proportion to its resources, is completely in the same spirit.

The topics selected are identical: nutrient requirements, an appraisal of the states of overnutrition (cardiovascular disease, obesity) and of undernutrition, a study of nutritional factors in infancy in relation to prevention of disease, toxicological aspects of food safety, and the dietary behaviour of individuals and communities. The spirits are ready and willing to work together; the means remain poor. The report from the UK is going to catalyse the timid French efforts.

To go even further, it would be desirable for Britain and France jointly to hold meetings and symposia on nutrition (once or twice a year to start with) at which points of view and communications of original work could be presented. The Nutrition Societies, the public bodies such as the MRC and ARC in the UK and the CNCERNA in France and the Nutrition Foundations could provide the impetus for such an arangement. The first step would be the formation of a working group, aware of the present deficiences of the subject and of the necessity to keep in touch all the time with the practical implications of food production and the maintenance of health and with fundamental research and the proper role of administrative and financial structures. The ultimate object would be to inspire research, and thus to develop a knowledge to use as a means in the service of the well being of the individual and the community. □

Marshall the energy

A profile of Eric Varley's Chief Scientist by David Fishlock

THE late Sir John Cockcroft, founder-director of the Atomic Energy Research Establishment, Harwell, in the early 1950s, was offered the post of Chief Scientific Adviser to the Cabinet. He accepted but retained his job as director of one of Britain's biggest research centres. Few today even recall that Cockcroft ever held the post in White-hall, so slight was his influence in the broader affairs of government. Dr Walter Marshall, present Director of Harwell, was expressly warned of this unhappy pecedent when, last summer, Mr Eric Varley, Secretary of State for Energy, offered him the post of Chief Scientist to his science-starved department. Marshall, however, would none-theless accept only on condition that he retained his job as Director of Harwell, Britain's—if not Europe's—most powerful energy research and development centre.

For Marshall, still a theoretical physicist at heart, whose own research earned him election into the Royal Society while still in his thirties, the most worthwhile job in the world is to be chief executive of this big science machine. Knowing that this is so is one important reason why morale is so high among the 5,000 staff of the Research Group of the UK Atomic Energy Authority (UKAEA), for which Marshall is the Board member responsible. But the Whitehall post, in which he would take a seat alongside the four existing deputy secretaries under Sir Jack Rampton in the Department of Energy, could expand vastly his influence over energy research and development in Britain. Coordination in this area, at a time when the emphasis was shifting from competition to conservation, would plainly be an important part of the role of government, as it became increasingly deeply embroiled in the activities of private as well as state-owned energy industries.

Even so, the twin jobs of advising both the Energy Secretary and his own boss Sir John Hill, Chairman of the AEA, could begin to work only if there existed a special relationship between Hill, as the Energy Secretary's adviser on the largest single sector of energy research and development, and Marshall, as his personal research adviser. But Marshall holds Hill in high esteem, not least for the support given Harwell during the difficult years when the laboratory was attempting to re-

build its reputation with a new an far more commercial approach, in fac of great scepticism from th scientific community as well as fror private industry. They devised the kin of formula that can work only betwee two people in closest rapport, to avoi mutual interference within each other parish in advising the Secretary c State for Energy.

Walter Charles Marshall, 43, joine Harwell from Birmingham Universit in 1954. Just six years later he becam Head of Theoretical Physics, and i 1966 Deputy Director, Two years late he was appointed Director of Harwe under Dr J. B. Adams, then Boar member for research of the UKAEA When Adams resigned the followin year to take charge of CERN's plan for the 300 GeV accelerator, Marshal was appointed Director of the Researcl Group of the UKAEA. In 1972 h became its Board member for research with full control of its research budge of £25 million.

By this time the prodigious energie of the burly young Welshman were al ready a legend—and not only at Har well. In 1969 Dr Alvin Weinberg, ther Director of Oak Ridge Nationa Laboratory, reflecting upon a perioc Marshall had spent with him in hi early thirties, remarked to me that had he remained much longer he would have taken over Oak Ridge.

But Harwell, by the mid-1960s, was presenting a challenge of a different kind. It was a research centre searching for a new mission. The basic physics and chemistry required to make nuclear weapons and lay the foundations for a nuclear power programme had been taken up by other laboratories, more firmly orientated towards engineering requirements. Harwell, some said, was becoming increasingly irrelevant. (*Nature* itself, in an editorial in October, 1967, argued that it should be closed down.)

One course of action open to the UKAEA was to cut the Research Group—then some 6,000 strong—back to a size commensurate with the mission remaining, mostly work for the fast reactor. This implied a reduction to about one-third of its size. The Government itself was well aware at the time of one drawback to this course of action, that those industries most urgently in need of new technical talent, such as the mechanical engineering industries, were the least likely to be enthusiastic about hiring redundant Harwell staff. Marshall and his colleagues, however, raised another important reservation—that a Harwell shrunk to one-third of its size simply would not be up to the very demanding business of nuclear energy research and development. So many questions remained unanswered about the fast

factor that nobody could possibly predict, when trouble struck, which of its vast array of techniques and talent would be needed

Marshall and his colleagues were vehemently opposed to either closure or a major reduction in strength As he put it in a paper published by the National Academy of Sciences last year "When a group of talented scientists and engineers have been brought together into a national laboratory and they have partially fulfilled their original mission, it seems to me unimaginative and wrong simply to disperse the ability national laboratories are a national asset, but they should be encouraged to change their orientation from time to time'

In other words, Harwell was seeking a new national mission to augment a diminishing role in nuclear fission The most obvious subject was nuclear fusion, but this had already been hived off to a new centre, the Culham Laboratory, while Marshall himself, once deeply into the physics of fusion, had turned to solid state physics because of frustration with the chaotic state of plasma physics in the mid-1960s

The mission conceived by Adams, Marshall and their colleagues was born of an awareness that "British scientists regularly produced good scientific ideas which led fairly rapidly to an improvement in the balance of payments of other countries but not of our own" (*The New Scientists*, OUP, 1971) At Harwell they had the skills, the equipment, and the proven ability to organise these facilities into effective problem-solving teams The mission they sought was the freedom to offer these facilities to industrial companies, on an exclusive basis, in an endeavour to help them to innovate

The Labour Government, well aware that Harwell was only the first of a number of national laboratories whose original missions were drawing to a close, looked kindly on Harwell's idea It even endorsed the proposal that Harwell should flout all traditions of British government-funded science and instead of making its research freely available to all-comers (as taxpayers) should work exclusively with chosen industrial partners With characteristic irreverence the idea was dubbed "the principle of maximum unfairness"

The difficulty, as Marshall records in *The New Scientists* was the "large number of arguments against the general idea, mostly drawn to our attention very forcibly by other people " Harwell's own attitude at the time was somewhat ambivalent, with many of the physicists and chemists opposed to the scheme, though the metallurgists, engineers, corrosion experts and so on were strongly in favour

Other opponents included industry itself, whose response was entirely unambiguous "Industry simply did not want us, and most people sought strongly to discourage us "

Marshall must take a major share of the credit for the success of the 'Harwell experiment' in which the portion of its income outside the Atomic Energy Vote has grown steadily to some £8 million last year, about 40% of the laboratory's budget

Marshall's industry-orientated research effort has, however, had its full share of disappointments and failures A prototype isotope-powered heart pacemaker failed in a patient A promising new method of desalinating sea water was turned down by the government as uneconomic when funds were sought for a demonstration plant Perhaps most disappointing of all was the decline and fall of carbon fibre

Last summer he shouldered the task of bringing the deductive logic of science to bear on the affairs of the new energy department In terms of influence the post had some obvious attractions, for the department is nominally responsible for an outlay on energy research and development which this year will total £120 million The post also provided a seat at the table of departmental scientific advisers counselling the Cabinet on scientific affairs

The obverse of the coin is that the research resources of the energy department are unquestionably undernourished Most of the £120 million is administered by the various energy industries and agencies, as the accompanying table shows As Marshall told the Select Committee on Science and Technology recently, his freedom for action in terms of research funds is restricted at present to a mere £200,000—the cash allocated last spring to set up the Energy Technology Support Unit at Harwell, which is appraising alternative energy options open to Britain

But as Chairman of ACORD, the Advisory Committee on Research and Development for the energy industries —traditionally an independent appointment but one Marshall insisted on taking himself—he can encourage a spirit of cooperation where once there was fierce competition, and of concern to conserve rather than to promote further use of particular fuels For the moment the emphasis is on technical services and what the application of research data already existing —perhaps garnered a decade ago—can do quickly to reduce energy consumption in industry

Outside ACORD, to which each of the state-owned energy industries will be submitting its annual report on research for appraisal this spring, the

Department of Energy is spending about £21 million in the current year on research contracts placed with Government and industrial laboratories Half of this sum is committed, however, to the Anglo-German-Dutch gas centrifuge project for uranium enrichment The only laboratory that came under the direct control of the department's Chief Scientist, namely the Safety in Mines Research Establishment, was transferred to the new Health and Safety Commission earlier this year

Several problems therefore loom large this spring for Dr Marshall First, he has a major part to play in preparing a paper for the Cabinet putting forward the case for a new phase in the development of the fast breeder reactor, far and away the most rewarding prospect in sight for research and development in energy conservation The new phase, however, will involve a sum of the magnitude of Britain's contribution to Concorde, for it must include a large scale demonstration power station using the sodium-cooled fast reactor

Second, he has to coordinate Britain's role in the energy research programme of the International Energy Agency which—largely, it is said, because of his own influence—blossomed abruptly in January with a series of proposals for multi-national ventures, with Britain taking the lead in coal technology

A third and possibly less tractable problem for Marshall is to create a research arm under his own control to the department's Offshore Supplies Office in Glasgow Only when thus equipped will the department feel competent to monitor what must surely be a rapidly burgeoning budget for offshore and seabed research and development contracts placed with other British laboratories

Fourth, he must strengthen his own scientific secretariat in the department, numbering merely 30 at present, and strongly orientated towards coal—a legacy of its origins in the erstwhile Ministry of Fuel and Power

A scientist on the Cabinet Office committee of departmental chief scientific advisers has remarked that Walter Marshall tends to raise eyebrows among his older and—in terms of Whitehall politics—much more experienced colleagues by starting his contributions "I don't know what you fellows think but at Harwell " Marshall is proud of the way he has rekindled the vitality of this research centre, transforming it into a brisk, businesslike organisation, second-to-none in the management of research projects Nobody would be very surprised if Walter Marshall now turned his top Harwell management loose on the problem of reshaping the nation's energy research and development effort

international news

In a move that will do little to enhance public confidence in environmental regulations and even less to restore public faith in technology, the Environmental Protection Agency (EPA) last week reluctantly decided to give automobile manufacturers at least another year in which to make further reductions in car exhaust emissions The agency really had little choice in the matter

The basis of the decision—which EPA Administrator Russell Train said last week is "the most unhappy decision that the agency has had to take since I have been here"—is relatively simple The catalytic converters which all American car makers have adopted to clean up exhaust pollutants may themselves pose a hazard to health, so the EPA has decided to see how serious the hazard may be before forcing even more widespread use of catalysts In short, although the catalysts seem to do a good job in oxidising hydrocarbons and carbon monoxide to less harmful compounds, they are also quite effective at oxidising sulphur dioxide to sulphuric acid, with the result that cars fitted with catalysts may be giving out harmful amounts of acid

The decision to put off enforcement of stricter regulations was politically painful because it now provides car

Car catalysts pose health problems

from Colin Norman, Washington

manufacturers and other industries with excellent propaganda material to use against any legislation which forces rapid introduction of clean-up technology When Congress belatedly forced the car companies to clean up exhaust emissions by putting a pistol to their heads in the shape of the Clean Air Act, the only technology available was the exhaust catalyst The car companies screamed that catalysts would be expensive, would increase gasoline consumption, and would simply be bad technology They begged for more time to develop other technologies, but Congress and the EPA decided that, on the basis of past performance, the car companies wouldn't use the time to develop other means of controlling exhaust emissions, and so forced them to use catalysts

As far as hydrocarbons, carbon monoxide and oxides of nitrogen are concerned, the catalysts have proved to be

extremely effective What's more, : spite of Detroit's warning to the co: trary, their introduction on many 197 model cars has even led to increase performance and efficiency Then can the sulphuric acid problem

It first came to light in the mi 1960s, but was disregarded as being 1 significant Then, in mid-1973 the prol lem was brought up again and give widespread publicity, and the EP, started a crash programme to dete: mine just how serious a hazard t health it might be The EPA found th; cars equipped with catalysts produce on average, 35 times more sulphuri acid per mile than cars without cats lysts Moreover, if an air pump is use to inject more air into the catalyst— device which would be required to mee stricter pollution control standards— then production of sulphuric aci would be about 70 times that of a ca without a catalyst

Although the amount of acid prc duced by automobiles would be only : tiny fraction of that produced by fac tories, the problem is that it would b concentrated along freeways and 11 cities

It wouldn't be so bad if there were : potential solution just around th corner, but there isn't None of th three possible solutions—removing sul phur from gasoline, putting a sulphu trap in the exhaust and improving cata lyst technology—provides a basis fo regulation, Train said last week I therefore seems at this stage that the only real chance of removing harmfu pollutants from car exhausts withou causing another health problem is fo the car companies to go for a com pletely different engine design, such a: the stratified charge engine But since Detroit produces 10 million cars a year it would be many years before the entire production line could be converted to a new technology

At least total amounts of automobile pollutants spewed into the atmosphere will continue to decline, even with the regulations frozen at this year's level, because older, dirtier cars will be replaced with cars equipped with 1975-level control devices But, since the fight over automobile pollution has been widely publicised, and because Congress and the EPA staked their hopes on the catalytic converter, the utter shambles that now reigns will be greeted with glee not only in Detroit but in a good many other corporate board rooms as well ☐

THE US Fish and Wildlife Service believes it has found a cheap and effective method for removing the potent teratogen dioxin from the herbicide 2,4,5-T If so, it would remove some, though not all, of the opposition to use of the herbicide in the United States, and it would also provide a solution to the problem of what to do with the 2 3 million gallons of dioxin-contaminated 2,4,5-T left over from the Vietnam war

The process is extremely simple According to an announcement from the US Department of Interior, all that is required is to filter the solution through coconut charcoal and to rinse the charcoal with acetone Tests carried out at the Fish and Wildlife Service laboratory at Columbia, Missouri, have shown that more than 99% of the dioxin in 2,4,5-T is removed by the method, and the government has applied for a patent for the process

Although removal of dioxin from 2,4,5-T would make the herbicide much safer, there is considerable doubt about whether or not 2,4,5-T itself is terato-

genic The herbicide is therefore still unlikely to get a completely clean bill of health from its critics

● After that embarrassing incident last year when President Ford had to withdraw the nomination of Andrew Gibson as head of the Federal Energy Agency when it became known that Gibson was drawing a large salary from the oil industry, Presidential appointees have been subjected to a thorough investigation before their names are sent to the Senate for confirmation Last week, however, Ford announced that four people have passed the test and are being nominated for top science posts They are Robert W Fri, to be Deputy Director of the Energy Research and Development Administration (ERDA), James L Liverman, to be Assistant Administrator of the ERDA for the environment, John Teem to be an Assistant Administrator of the ERDA for advanced energy systems, and Richard C Atkinson to be Deputy Director of the National Science Foundation

THE Medical Research Council is to set up a new unit for research in clinical oncology and radiotherapeutics at Cambridge, at the same time, the Cancer Research Campaign has provided an endowment of £275,000 to establish a new university chair of clinical oncology Professor Norman Montague Bleehen has been appointed Honorary Director of the MRC Unit and Professor of Clinical Oncology He will take up both posts at the beginning of the academic year in October The unit officially starts operation in October and will be provisionally housed at the new Addenbrooke's Hospital It is hoped that purpose-built accommodation can be provided within five years Professor Bleehen is at present Professor of Radiotherapy at the Middlesex Hospital Under his direction, the new unit's general aim will be to improve the results of current methods for the treatment of cancer and to develop improved methods of measuring the clinical and biological response of tumours to treatment The programme will include investigations on lung cancer, rectal cancer and osteosarcoma, with particular reference to occult metastatic disease Staff at the new unit will collaborate closely with other workers there with oncological interests In particular, it will provide a clinical link for the laboratory research now being carried out in Cambridge by a Tumour Biology Group led by Dr Sydney Brenner at the Laboratory of Molecular Biology

The new chair in clinical oncology is the fourth to be funded by the Cancer Research Campaign Chairs have already been set up at the Institute for Cancer Research in London and at the universities of Manchester and Glasgow A fifth is to be established shortly at the University of Southampton, and when complete the scheme will have cost about £3 million The Cancer Research Campaign is the largest supporter of research into cancer, including leukaemia, in the UK and in 1974 awarded grants totalling more than £4 5 million

● In this year's list of prizes and medals awarded by the Institute of Physics, afficionados of the entertaining experimental demonstrations at the Royal Institution lectures will be glad to see the award of the Bragg Medal and Prize to Mr W A Coates "for his contribution to education through the design and execution of demonstrations illustrating the many lectures held at the Royal Institution" Mr Coates first joined the Royal Institution in 1948 as a technical research assistant on X-ray diffraction experiments and became Senior Experimental Officer in charge of preparation and production of teaching demonstrations under the directorship of Sir Lawrence Bragg

After more than 10 years organising the demonstrations, which Mr Coates admits he embarked on with some reluctance when Sir Lawrence asked him to help with the lectures for schools started in the late 1950s, Mr Coates still thoroughly enjoys a job which can have its unexpected hazards Recently, after setting up a demonstration for measuring the potential of an electric eel, an ugly customer which can give a shock up to 400 volts, he returned from a meal to find the tank empty and the eel gone Luckily it had landed on a rubber mat nearby and so was returned to its tank with no further mishap, the demonstration was a complete success

Round Britain

● The publication this week of the latest annual report of the Science Arm of the Agricultural Development and Advisory Service (ADAS) of the Ministry of Agriculture (HMSO, £5 50) should prove a good reference book for agricultural research scientists looking for sources of useful but often unpublished data, and of problems which would repay further research

In their role as scientific troubleshooters to the agricultural industry ADAS has amassed a unique collection of field and experimental data (which of its nature is usually not publishable in the conventional scientific literature) on a wide variety of agricultural problems ranging from heavy metal contamination of sewage sludges used as fertilisers and water pollution by agricultural wastes, to the optimum feed formulations for cattle As well as highlighting problems in need of further research, such data will prove invaluable in drafting pollution legislation, for example

Much of this work had previously been hidden in internal reports but with the formation of ADAS in 1971 from several of the ministry's advisory services, it was felt that the work could be usefully brought to the attention of a wider audience Formal links between ADAS and the Agricultural Research Council have also been strengthened recently with the formation of the Joint Consultative Organisation for Agricultural Research Policy, on which ADAS is represented

Britain's entry into the EEC has also involved ADAS in the collection and evaluation of technical information and advice for the negotiating teams in Brussels trying to harmonise the many regulations concerning agricultural practice and produce throughout the community—for example those dealing with the antibiotic content of animal feeding stuffs and the tests used to determine the quality of milk and milk products The service has also been involved in the analytical tests to determine standards of produce submitted to the Intervention Board for Agricultural Produce

● Professor Jack Meadows, of the University of Leicester, has been given a £16,000 grant by the British Library to study how scientific ideas get to the mass media and how they are used by television, radio, newspapers and magazines Part of the grant will be used for research into the way in which journals monitor research papers and why some work is rejected and some accepted in a situation in which the journal is receiving more 'good' papers than it can publish

This work stems from a study in the United States by Merton and Zuckermann, who monitored the rejection rate of the leading specialised journals over a range of subjects and found a definite correlation with subject Journals of the physical sciences had lower rejection rates compared with biological journals, and the rate was even higher for psychology and learned journals of the humanities The American workers also found that the rejection rate was positively correlated with the degree of disagreement among the referees

Professor Meadows and his team hope to extend this type of investigation into the British system, as the influences at work may well be different from those in the United States He hopes to find out in detail what happens in the refereeing process, and whether some of the myths surrounding refereeing are in fact justified Needless to say this type of investigation must be organised so that the confidentiality of the refereeing system is maintained In tune with the times, the team will also investigate how the economic situation, as reflected in decreases in the number of journal pages, has affected the refereeing process by perhaps making it more stringent

● Much head-scratching and pen-chewing in Cambridge these days Professor J W Linnett, Vice-Chancellor and Professor of Physical Chemistry, is worried that university research is being poor-mouthed by certain 'ladies and gentlemen' So he has circularised departments to enquire of any "useful research of clear value in the relatively short term" If the right sort of stuff comes up he then intends to alert ministers, MPs, newspapers and industrialists to the merits of the place

LAST year Israeli farmers produced 78% of the foodstuffs consumed in Israel, as well as exporting enough agricultural products to pay for most food imports Now, with the active assistance of local researchers, they are attempting to reduce further Israel's dependence on imported food products, particularly commodities like wheat and sugar which have gone up in price by hundreds of per cent in recent years

Research has already been a key factor in raising the average yield of wheat from some 900 pounds an acre in 1950-55 to more than 1,600 pounds an acre today, with yields of 6,000 pounds an acre common in more fertile parts of the country Current studies could well increase output even more

Some of the most interesting work is being carried out by Moshe Feldman and Dan Atsmon, of the Weizmann Institute, who are using sophisticated cytogenetic techniques in an attempt to transfer valuable characteristics from native Israeli wild wheat to standard wheat varieties being grown here and elsewhere

Since this area, long known as the Fertile Crescent, is the cradle of wheat farming, it naturally abounds in wild varieties And although these are not satisfactory for modern farming themselves, they nevertheless manifest some very interesting characteristics worth "stealing"
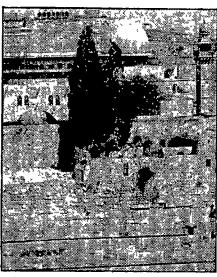
Drs Feldman and Atsmon are paying special attention to a species from the Negev Desert (*Triticum longissimum*), which is at home in an area with as little as 25 cm of rainfall annually, tolerates brackish water, and grows and matures quickly—so much so that, were cultivated wheat to ripen as fast, it would be possible to plant and harvest two (irrigated) crops a year

The Weizmann scientists have isolated seven individual chromosomes of the Negev wild wheat, including the one responsible for its early maturation Now they are attempting to incorporate selectively these valuable chromosomes into standard wheat varieties, which should make it possible to increase yields in well watered areas, and also permit wheat to be grown in other areas where sparse rainfall has so far prevented its cultivation

More land will likewise be given over to the growing of sugar beet, while research continues in an attempt to develop other economically viable sources of sugar and sugar substitutes Ministry of Agriculture studies have indicated, for example, that at present price levels it is worth making sugar from locally grown sorghum

Meanwhile scientists at Ben-Gurion University of the Negev, led by Pro-

fessor Jaime Wisniak, are concentrating on sugar substitutes, including xylitol So far produced on a commercial basis only in Japan, xylitol is derived from vegetable residues such as cotton seed hulls, corn and other seeds containing cellulose It has the same crystalline form as sugar and is actually 10% sweeter Since it does not require insulin for metabolisation of body material, it can safely be used by

Letter from Israel

from Nechemia Meyers

Western Wall   to weed or not to weed?

diabetics Wisniak proposes that Israel produce xylitol on a large enough scale to meet domestic requirements and to allow for exports

● Israeli scientists studying plant life are involved not only in solving the country's food problems but also some of its religious problems Namely, they are being asked by religious authorities whether or not weeds should be pulled out of the sacred Western Wall in Jerusalem's Old City

Diametrically opposed rulings have been issued by Israel's constantly feuding Chief Rabbis, Shlomo Goren and Ovadia Yosef Ashkenazic Chief Rabbi Goren declared that the plants could not be pulled out since they are symbolic of the destruction of the Second Temple (in 70 AD) and the longing of Israel for redemption Sephardic Chief Rabbi Yosef ruled that they could be pulled out, particularly as they might crack the Herodian-period stones

The man who asked the Chief Rabbis for their opinions in the first place, Rabbi Dov Perla of the Religious Affairs Ministry, has now turned to a botanist to determine—for the first time in the Wall's 1,900-year history— whether or not the weeds are likely to

cause damage

● Religion, or more properly religious folklore, provided the name for Israel's largest locally built computer, the Weizmann Institute's Golem The original Golem was a legendary automaton created by a famous Prague rabbi in the Middle Ages and, like Mary Shelley's Frankenstein, it eventually turned on its creator

The legend came to mind last week when 30,000 Israeli primary school teachers declared what may have been the world's first strike against a computer Their one-day walkout was called to protest about difficulties with the Ministry of Education's computer which have caused long delays in the payment of salaries for the past 16 months

Computing teachers' salaries does present special problems, as 18,000 changes must be taken into consideration every month But these problems could easily have been solved if it had not been for the human factor

Trouble began in the Ministry of Education's Data Processing Department some four years ago when it became clear that the rented IBM 360/40 then being used to calculate salaries was no longer able to handle the work load Department employees suggested that it be replaced by a higher capacity IBM machine, which, coming from the same company, would ease conversion problems The ministry decided instead to purchase a Burroughs computer because it was $250,000 cheaper and because, according to benchmark tests carried out in the USA, it was able to perform tasks required by the ministry about 15% faster

Preparations for conversion to a Burroughs computer were carried out in the department while regular work was done on the old IBM Difficulties arose, but only became really serious when call-ups because of the Yom Kippur War took away 80% of the data processors By the time they returned there was near chaos in the salary section, prompting employees to argue that only the introduction of a new IBM could save the situation When the ministry remained unmoved, 46 of the 47 people in the Data Processing Department handed in their resignations and, according to Education Ministry Director General Elad Peled, left without passing on vital information about tape codes for salary calculations to their replacements (provided by the local representatives of the Burroughs Company)

Peled charged his former subordinates with sabotage, they, in turn, charged him with libel But all this is of little interest to teachers, who merely want their salaries on time

'lood studies rom NERC

om Angela Croome

'HE engineer designing a dam or a eservoir, or the city planner contem- lating urban development on river mbankment or flood-plain is in the ands of the hydrologist and to some xtent the meteorologist Britain's Insti- ution of Civil Engineers (ICE) grasped his fact in the 1930s after a major re- iew following the last failure of a dam n Britain—happily now 50 years ago which is something of a record) The nstitution has pressed ever since for a omprehensive applicable prognostic pproach enabling engineers to design vith confidence a structure which vould last Britain's civil engineers btained this document last week in he form of the Natural Environment Research Council's *Flood Studies Re- ort*

A draft code of practice closely ssociated with this report is coming ut for discussion within a few weeks, nd from some time in 1976, when dopted in final form, any civil engineer n Britain who does not follow it will e guilty of professional negligence In May the ICE is calling a meeting in London to examine in detail the report nd the code of practice, their validity, nd their relevance outside Britain
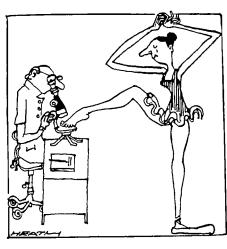
The influence of the report is likely o be great and much more widespread han its original terms of reference might suggest It is the most compre- hensive attempt that has been made anywhere to tackle the problem of flood estimation and, in the opinion of the International Commission on Large Dams, it is "head and shoulders" above all others The ICE is already prepared to say that as a result of its findings many flood-protection schemes will have to be revised

The study has involved nearly five years of work by a multidisciplinary team based on and led by a staff of 15 recruited by the NERC Institute of Hydrology, and assisted by a six-man group of Meteorological Office scien- tists The statistical analysis of cor- related rainfall and runoff records on the widest possible scale forms the core of the study This has involved collect- ing an archive of 9,300 'station-years' of records (from the 1,200 stream- gauges in the UK) plus a further 1,700 'station-years' from 110 Irish gauge- stations Records from 6,000 rainfall stations were also included

The result in practical terms is that for any area of 500 km² in Britain, a reliable estimate of the mean annual flood level and its frequency can be obtained (In other terms, the report amounts to the most comprehensive

available textbook on hydrological sta- tistics—for all the data is tabulated there as well as the mathematical rela- tionships derived from them)

Basically, there are two methods now available Where there are gauge- station records for a given river basin, a statistical method is presented enab- ling the practitioner to get specific answers to his questions—how high is the maximum flood level he must build for over, say, 10,100 or 1,000 years, and how often is it reached This can be derived either from records of the annual maximum for the area or from data on the peaks above a threshold level Where only rainfall records exist —that is, in an ungauged river basin— the run-off characteristics of a site can be estimated, given information on four characteristics of the catchment, namely, slope, soil, land-use and area As rainfall records are usually more extensive and go back further, the sec- ond method may be more easily applic- able outside Britain but it can only be reliably applied in other cool or tem- perate regions □

THE results of a 15-year survey, car- ried out on more than 100 pupils of the Kiev School of Choreography, has revealed significant modifications in the toe structure of ballet students, these are put down to the increased loads imposed on the toes when dancing on the point or demi-pointe The study, carried out by biologist Vladislav Peli- penko and based on regular X-rays of the toes during the course of 15 years of training and dancing, showed that within a short time the bones undergo significant thickening of the outer layers and restructuring of the inner layers On the basis of these measure- ments, Pelipenko has compiled tables which, he claims, will provide a basis for the selection of new pupils and a means of forecasting their future per- formance The results of these experi- ments, he suggests, can also be applied to draw up training schedules for gym- nasts and athletes, who also experience unusually large loads on the toes □

Science writers and government policy

from David Spurgeon, Ottawa

PROFESSIONAL science writers and scientists co-exist for the most part under conditions of uneasy truce Thus it is refreshing when one side finds good things to say about the other Just such an occasion was the fifth annual science writing seminar of the Cana- dian Science Writers' Association, held on February 24 and 25 at the Univer- sity of Toronto The welcome by Dr George Connell, Vice-President Re- search and Planning at the university, was positively fulsome

The reason, basically, was that Dr Connell felt that "active and well in- formed men and women of the press, radio and television" were to a large extent responsible for influencing the federal government to reverse the re- cent downward trend in research funding

"For the first time in six years", said Dr Connell, "the levels of support through the [research] councils will balance inflation "

Dr Connell recalled that the buying power of the average grant from the National Research Council (NRC) in the physical sciences, the biological sciences (excluding health sciences) and the engineering sciences decreased by 7% between 1970 and 1974

"Early in 1974 a group of medical scientists and well informed laymen decided to present to the public an account of the benefits of medical re- search and the difficulties which have impeded its progress," Dr Connell said "The response of the press, radio and television to this initiative was remark- able in Toronto the daily newspapers provided coverage which was remark- able both for its quality and quantity The activity in other cities across the country was also notable "

The outcome, the vice-president said, was that, in the main estimates tabled the week before the seminar, the gov- ernment proposed to spend $48 4 million through the Medical Research Council (a 13% increase over the 1974-75 esti- mates), NRC estimates for scholarships and grants increased from $69 3 million (1974-75) to $81 7 million (1975-76) and the Canada Council's increase was from $23 9 million to $27 7 million

The usual discussion took place of the difficulties of understanding and translating scientific jargon, but it was left to a French Canadian writer to put things in a suitably Canadian perspec- tive he said that being a French speaker was a much bigger communi- cation problem than all the rest, be- cause French-speaking scientists in Canada are so hard to find □

correspondence

Nutritional landmark

SIR,—Reports prepared by expert committees on specific areas of nutrition are frequently issued, notably by the Food and Agricultural Organisation and the World Health Organisation It is, however, an event of significance when a comprehensive survey of food and nutrition research in a single country with a strong tradition of scientific and medical investigation is assembled by a large team representative of the available experts It is indeed a considerable achievement to have synthesised such a readable report from the documents provided by the various working parties, to one of which I was a minor and temporary contributor The product is a landmark document, which appears at a time when the bridge between basic research and applied research in the national interest must be strengthened in the interests of survival Food and nutrition research needs to attract original minds trained in the basic sciences This document should provide the points of contact It is thus entirely proper that the committee responsible for assembling the report should have included Sir Douglas Black, Chief Scientist to the Department of Health and Social Security and responsible in Britain for implementing contracts in areas of applied research

I write this comment because a petulant and partly irrelevant review appeared in *Nature* (January 10) castigating the report because of insufficient emphasis on social nutrition, and ridiculing the priority areas for research identified in the report On the contrary, the report correctly identifies and summarises current knowledge in numerous areas of nutritional research that are of great importance to human health and survival the role of nutrition in vascular disease, diet and cancer, dietary fibre and the spectrum of diseases in a population, the aetiology of osteoporosis, nutritional aspects of renal disease, to name some in only one section of the report The current problems in food and nutrition research are numerous, important and challenging Further research into the areas identified in this comprehensive document should allow governments to plan research strategies, to test new food sources and to monitor the population more efficiently Finally, the present disreputable status of nutrition research among basic scientists and the medical profession will also be materially mitigated by the vigorous well-founded directions indicated in the report

H V MUNRO
Massachusetts Institute of Technology Cambridge, Mass

Re-use of vials

SIR,—In the February 27 issue, Professor Born rightly drew attention to the need to minimise waste in laboratories and cited as a specific example the destruction of scintillation vials after being used only once It may be of general interest, therefore, that in this department we have for a number of years successfully re-used vials without risking contamination or any of the other problems which Professor Born mentioned The procedure we have adopted involves placing the radioactive sample and scintillation fluid in an ordinary $5\,cm \times 1\,2\,cm$ diameter specimen tube that is inserted into the vial for counting This simple method has the following advantages (1) the vials themselves are not contaminated, (2) the volume of scintillation fluid is only 2 ml, thus reducing costs, (3) the specimen tubes are very much cheaper than the scintillation vials They may be either discarded or used for other purposes after normal cleaning

The method works particularly well for non-aqueous samples—for example, materials on membrane filters or papers —since in these cases there is no reduction in either the counting efficiency or the amount of labelled material used in the assay

H R V ARNSTEIN
Department of Biochemistry,
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Science and technology

SIR,—In his paper on the changing relationship between science and technology (August 23, 1974) Langrish quotes evidence from previous citation studies which, he asserts, support his views that "British university research has little impact on British industry" We wish to challenge the view that generalisations can be made across all fields of scientific endeavour on the usefulness or otherwise of university research to industry on the basis of the evidence provided by citation analysis

On the assumption that industrial reviewers will cite any universit research which they consider to be c relevance to industrial activity, Langris chose seven review articles written b British industrial chemists in the 196 volume of *Reports on the Progress c Applied Chemistry*, and found that th papers which the reviewers considere most relevant to technological develop ments in the seven areas were largel of non-university origin The area chosen were phenolic resins, micro biological techniques in manufacture water treatment, plastics, polyolefins synthetic detergents and poultry an eggs In only one area (microbiologica techniques) was the influence of uni versity research dominant, and thi indicated to the author the importanc of the universities in generating nev techniques for industrial uptake

We suggest, however, that a citatior study such as this fails to take intc account the fact that it is much more likely that there will be university research activity in the area of micro biological techniques, for example, than in water treatment, and that there may be certain major areas of scientific enquiry in which the institutional sources of citations are quite different from the sources found in the disparate subject-areas used as samples by Langrish In particular, we suggest that the conclusions reached in citation studies of the physical sciences do not have general validity and, in order to demonstrate this, we carried out a study of the institutional sources of citations in five biologically oriented review articles in *Reports on the Progress of Applied Chemistry* All the articles were concerned with well established areas of scientific enquiry, and showed that in terms of citations, the universities outstripped every other sector

One should be wary of generalisations about the industrial utility of university research which rely on evidence from citation data Langrish has stressed the implications of his citation data for policy makers in government and industry, and the fact that we have been able to obtain results which differ so widely from his raises serious doubts as to the validity of the technique of citation analysis in the determination of the relationship between science and technology Policy decisions cannot and should not be made on the basis of such dubious evidence

G PRAGIER
J RONAYNE
Brisbane, Australia

news and views

Viruses associated with human leukaemia

rom Robin Weiss

'HREE new reports strengthen the pinion that there is a C-type virus ssociated with human leukaemias /Iak and his colleagues at the)ntario Cancer Institute (*Proc natn lcad Sci U S A*, **72**, 623, 1975, and **1**, 4336, 1974) describe the appearance f virus-like particles in medium from hort-term cultures of leukaemic bone narrow cells Cultures of bone mar-ow from normal individuals did not elease particles, but cultures from both ymphocytic and myelocytic leukaemic patients did so, when the bone marrow vas taken either at blast cell crisis or n remission Mak *et al* have not been ble to demonstrate budding particles, lthough the incorporation of tritiated iridine into the RNA of the particles ndicates that viral RNA synthesis)ccurs in culture The virus-like par-icles have the buoyant density, reverse ranscriptase and 60–70S RNA typical)f animal C-type viruses, and mole-cular hybridisation studies indicate that it is related to woolly monkey ymphosarcoma virus and to Rauscher virus In contrast to their bone marrow cultures, Mak *et al* found that cultures of peripheral white blood cells taken from leukaemic patients do not release virus-like particles The Toronto group seem however to be unaware of an earlier report by Kotler *et al* (*Nature new Biol*, **244**, 197, 1973) that white blood cells from patients with lymphocytic leukaemia can be induced to release very similar particles when cultivated in medium lacking arginine

Gallagher and Gallo at the US National Cancer Institute (*Science*, **187**, 350, 1975) describe the production of virus from cultures of three myelo-blastic cell lines from the peripheral blood of a patient with acute myelo-genous leukaemia For the first time these authors show really convincing electron micrographs of C-type virus particles, including budding forms The virus found in the medium of the myeloblast cultures possesses all the physical and chemical properties that characterise C-type viruses, and the reverse transcriptase activity is speci-fically inhibited by antibodies prepared against the reverse transcriptases of gibbon and woolly monkey viruses

Gallagher and Gallo did not observe virus production in white blood cells freshly isolated from leukaemic patients, but on prolonged cultivation of the myeloblasts from several cases of acute myelogenous leukaemia, virus began to be released from cultures derived from one 61-year-old patient Perhaps the most notable advance is not the appearance of the virus itself, but is the ability to maintain long-term, exponentially proliferating cul-tures of myeloblasts The growth of the myeloblasts depends on a conditioning factor produced by a human embryo cell strain Gallagher and Gallo believe that the conditioning factor, which has not yet been characterised, is neces-sary both for myeloblast growth and virus release They are confident that the virus arises directly from the leukaemic cells, since neither virus nor viral components were detectable in the embryo cell strain or the filtered, onditioned medium

Scherr and Todaro at the US National Cancer Institute (*Science*, **187**, 855, 1975), using material generously provided by Gallo and Gallagher, now report that antigens related to a major structural protein (p30) of the woolly monkey and gibbon C-type viruses are present in the white blood cells of five patients with acute leukaemia

While these new findings mark a significant advance in the search for human leukaemia viruses they come as little surprise Over the last three years evidence has steadily mounted, largely from Spiegelman's and Gallo's laboratories, that a viral entity is associated with human leukaemic cells A DNA polymerase with the properties of viral reverse transcriptase was regularly detected in human leukaemic cells (*Nature new Biol*, **240**, 67 and 72, 1972) The enzyme was associated with high molecular weight RNA in a cytoplasmic particle but no complete, cell-free virus particle could be detected Later, Todaro, Gallo and Gallagher (*Nature*, **244**, 26, 1973, *Proc natn Acad Sci U S A*, **71**, 1309, 1974) showed that the enzyme activity of the cytoplasmic 'particles' was specifically inhibited by antisera raised against the reverse transcriptases of gibbon and woolly monkey C-type viruses Hybridi-sation studies by Baxt, Spiegelman and

colleagues (*Proc natn Acad Sci USA*, **69**, 3737, 1972, **70**, 2629, 1973 and **71**, 2853, 1974) indicated that DNA complementary to the particulate RNA was present in human leukaemic cells but was not detectable in normal white blood cells, not even in two cases of identical twins to the leukaemic patients Gallo's group (*Proc natn Acad Sci U S A*, **70**, 3219, 1973 and **71**, 3177, 1974) have compared the homology of the 'viral' RNA of human leukaemic cells to a variety of animal C-type viruses and have found that it is most closely related to the woolly monkey lymphoma–sarcoma virus complex

It was therefore predictable that the human viral particles isolated by Gallagher and Gallo and by Mak *et al* would prove to be related to the woolly monkey virus, the human leukaemic p30 proteins described by Scherr and Todaro are indistinguishable in a radioimmune competition assay from the p30 of the woolly monkey virus

Oncologists have become justifiably sceptical of new claims for the isola-tion of human tumour viruses, since some highly publicised announcements of human viruses in the past were found on subsequent analysis to be contaminating animal viruses Clearly it is going to be difficult to prove that these latest isolates are not a result of contamination with laboratory stocks of woolly monkey virus Nevertheless, there are several telling points against contamination First, it is clear that freshly isolated human leukaemic cells that have not been maintained in culture carry the reverse transcriptase, p30 protein, and some RNA sequences related to the woolly monkey virus Second, Mak's virus-releasing bone marrow cells were all short-term cul-tures maintained in a laboratory where woolly monkey virus was not pro-pagated Third, the virus produced by Gallagher's long-term cultures appeared in three sublines that were maintained separately from the first passage on-wards But it does seem strange that a human virus should have such a close affinity to a virus complex isolated from a New World monkey To my mind, it is more likely that at least one component of the woolly monkey virus complex was acquired from man,

for it represents a single isolate from a pet monkey (Theilen *et al*, *J natn Cancer Inst*, **47**, 881, 1971) Perhaps the long sought human virus has been in our laboratories for the past four years

The human leukaemia 'virus' does not seem to be endogenous because homologous proviral nucleotide sequences have not been detected in DNA extracted from normal human tissues, not even in Baxt's identical twin studies referred to already There is however some recent evidence for an endogenous human virus related to RD-114 and baboon viruses (Strand and August, *J Virol*, **14**, 1584, 1974, Scherr and Todaro, *Proc natn Acad Sci U S A*, **71**, 4703, 1974) This virus does not seem to be closely related to the woolly monkey virus and its expression is not particularly associated with leukaemia or with other neoplasms, but rather with systemic lupus erythematosus In cats, for instance, the infectious, leukaemia-causing virus has very little homology with the endogenous viral genome Could a human leukaemia, therefore,

be acquired by infection, as in cats and chickens?

There is no epidemiological evidence that leukaemia is contagious, except for one recent report by Schimff *et al* (*Lancet*, **i**, 124, 1975) of possible clusters in incidence of leukaemia of various kinds by social contact in a small rural community in West Virginia If, however, leukaemia were a rare and late consequence of a very widespread and common infection it would be difficult to obtain epidemiological evidence for infection Indeed this was the case with feline leukaemia before the virus was isolated and exploited as a serological tool In concluding their paper, Scherr and Todaro state that "the isolation of complete C-type viruses from human leukaemic cells should greatly assist in evaluating their etiologic role in the disease" A number of laboratories are now attempting to propagate the viruses or particles isolated by Mak and by Gallagher and Gallo while further, independent isolates are eagerly awaited

accumulate Straight chain acids of th type have been found by Barley *et a* in rat glial cells cultured in vitami B_{12}-deficient medium (*J biol Chem* **247**, 4270, 1972) More recently simile abnormal fatty acids together with branched chain 17-carbon version wei found in autopsy tissue from a chi: who had extremely high levels of urii ary methylmalonic acid probably as result of a congenital inability to coi vert vitamin B_{12} into its coenzyme forn (Kishimoto *et al*, *J Lipid Res*, **14**, 6! 1973) And, most exciting, Frenkel h; demonstrated 15- and 17-carbon fati acids in peripheral nerve biopsies froi cases of pernicious anaemia (*J cli; Invest*, **52**, 1237, 1973)

It is probable that the straight-chai fatty acids are formed when propriony coenzyme A substitutes for acetyl cc enzyme A as a substrate for fatty aci synthetase and the branched-chain fatt acids are made when methylmalony coenzyme A substitutes for malony coenzyme A The big question tha remains is whether the accumulate abnormal fatty acids are really th cause of the neural lesion associate with vitamin B_{12} deficiency and if sc how? Even the more impressive of th above studies have their shortcoming; How closely do cultured rat glial cell really relate to human spinal cord ii *vivo*, particularly considering that th rat does not develop neurologica problems when depleted of vitamin B_{12}' Why do children with congenita methylmalonylaciduria seldom, if ever exhibit the same neurological lesions a: in pernicious anaemia? And are th abnormal fatty acids found in the peri pheral nerves in pernicious anaemi also found in the spinal cord where th demyelination occurs? Nevertheless th similarity of the findings in each stud are impressive enough for the prob lems to be pursued The availability o the bat as a model for the neurologica changes of human vitamin B_{12} defi ciency should considerably assist the pursuit

For those who cannot work with bat: there are always bugs Work continue: apace on the metabolism of vitamir B_{12} in bacteria, if for no other reasor than the pious hope that there are un discovered vitamin B_{12}-dependent en zymes which are essential for the normal functioning not only of bacteria but of man as well Thus methionine synthetase, the only known vitamin B_{12}-dependent mammalian enzyme apart from methylmalonyl-CoA mutase, was discovered in *Escherichia coli* But it should not be forgotten that ribonucleo tide reductase, found in *Lactobacillus leichmann* as a vitamin B_{12} requiring enzyme, was at one time the great white hope for the biochemical basis of the megaloblastic anaemia of human vita min B_{12} deficiency Although the en-

Vitamin B₁₂ in bats and bugs

by Peter Newmark

THE neurological lesion which often accompanies human pernicious anaemia has no certain biochemical explanation One of the hindrances to progress has been the lack of a suitable animal model Though vitamin B_{12} deficiency (the cause of pernicious anaemia) results in both megaloblastic anaemia and demyelination of the spinal cord in humans, no common experimental animal placed on a vitamin B_{12} deficient diet has shown a consistent neurological lesion comparable to that in man But according to the report by Green *et al* in this issue of *Nature* (page 148) the Egyptian fruit bat may fill this gap

The success of Green *et al* may have had its origin in the natural diet of the bats This is vegetarian and therefore ostensibly devoid of vitamin B_{12} Green *et al* suggest that wild bats obtain the vitamin by consuming both stagnant water and insects contaminating the dietary fruit Both sources could be excluded in the laboratory diet of peeled fruit and tap water Within 7 months the bats had difficulty in climbing and flying associated with histological changes in the spinal cord reminiscent of the demyelination seen in human vitamin B_{12} deficiency

If this model fulfils its promise it will have appeared at an opportune moment for exploitation because recent evidence has at last suggested a plausible bio-chemical link between vitamin B_{12} deficiency and demyelination The link

is based on the requirement of a vitamin B_{12} coenzyme for methylmalonyl-CoA mutase and the consequent accumulation of methylmalonyl coenzyme A in vitamin B_{12}-deficient tissues Excessive methylmalonyl coenzyme A, and its precursor propionyl coenzyme A, could interfere with the normal metabolism of the fatty acids of the myelin sheath, resulting ultimately in demyelination The evidence that now makes this a distinct possibility is several fold

Clearly there is some rebalancing in the fatty acid metabolising enzymes of vitamin B_{12}-deficient tissues Frenkel *et al* (*J biol Chem*, **248**, 7540, 1973) have demonstrated increased activities of both acetyl-CoA carboxylase and fatty acid synthetase in the liver of vita-min B_{12} deficient rats, possibly in res-ponse to the inhibition of both enzymes by methylmalonyl coenzyme A The increase, at least of fatty acid synthe-tase, seems to be the net result of markedly increased synthesis of the enzyme together with a less dramatic enhancement of its degradation (Frenkel *et al*, *Archs Biochem Biophys*, **162**, 607, 1974) It is not, however, altogether obvious how to translate such changes into likely effects on the neural tissue

Much more impressive therefore have been demonstrations of accumulated abnormal fatty acids in neural tissue accompanying vitamin B_{12} deficiency In particular it is 15-carbon and 17-carbon saturated fatty acids that

zyme is not confined to *Lactobacillus* (Hamilton, *J biol Chem*, **249**, 4428, 1974), there is now little doubt that in mammalian tissues it is replaced by a vitamin B₁₂-independent version

Ethanolamine deaminase is indubitably not a mammalian enzyme It is found in clostridial bacteria, is vitamin B₁₂ dependent and has been studied in great detail by Babior (for example *J biol Chem*, **249**, 4537, 1974) Chang and Chang provide evidence on page 150 of this issue for the presence of the same enzyme in *Salmonella typhimurium* and perhaps also in other enteric bacteria The enzyme, like the clostridial one, is probably induced as a response to the supply of ethanolamine as the sole source of carbon and nitrogen together with vitamin B₁₂ Exactly why these bacteria should have the potential to grow on such an unnatural duo of nutrients is puzzling

Hormonal control of spermatogenesis

from R V Short

IN reviewing the hormonal control of mammalian spermatogenesis, Steinberger commented that "knowledge of the biochemical parameters of the hormonal action on spermatogenesis is too meagre even for a limited attempt to formulate a working hypothesis" (*Physiol Rev*, **51**, 1, 1971) Research so often seems to be at a standstill, that it is refreshing to record a number of important advances in our understanding of spermatogenesis in the last four years

There is now growing evidence from clinical (Franchimont *et al*, *J clin Endocr*, **34**, 1003, 1972, De Kretser *et al*, *J clin Endocr*, **38**, 787, 1974) and laboratory studies (Setchell *et al*, *J Endocr*, **62**, 675, 1974) that the pituitary gland is somehow aware of the number of spermatozoa that the testis is producing "Inhibin", a substance first postulated by McCullogh in 1932 (*Science*, **76**, 19, 1932), really seems to exist It is apparently produced by the germinal cells of the testis, and acts centrally to suppress the secretion of follicle stimulating hormone by the pituitary gland A number of laboratories throughout the world are actively working on the purification and identification of this new testicular hormone, and success seems near at hand

We are also beginning to get a clearer idea of the role of the Sertoli cells in the control of spermatogenesis These cells, which line the seminiferous tubules of the testis, are in intimate association with the germ cell line at all stages of their development, from spermatogonia to spermatozoa This close proximity suggests that there could be an important functional interrelationship between germinal and somatic cells, but hitherto we have lacked any biochemical understanding of the nature of this relationship Thanks to the collaborative work between Drs Hansson (Oslo), Ritzen (Stockholm) and French (Chapel Hill, North Carolina), we can now begin to understand exactly what is happening

It seems that the Sertoli cell is stimulated by follicle stimulating hormone to secrete an androgen-binding protein with a high affinity for testosterone and its biologically active metabolite, dihydrotestosterone (*Nature new Biol*, **246**, 56, 1973, *Nature*, **250**, 387, 1974) As Hansson and his colleagues point out in this issue of *Nature* (page 145), testicular androgens are known to be the principal stimulus for spermatogenesis, they seem to be required for almost all the stages of meiotic division (Steinberger, *Physiol Rev*, **51**, 1, 1971) In normal circumstances, luteinising hormone secreted by the pituitary gland acts on the interstitial or Leydig cells of the testis to induce testosterone secretion Though much of this hormone then enters the systemic circulation, the intratubular androgen binding protein may be a vital link in the chain, enabling some hormone to be captured and concentrated within the lumen of the seminiferous tubules, and subsequently transported to the germ cells where it will stimulate meiosis

It has long been known that it is possible to maintain complete spermatogenesis in hypophysectomised animals if they are placed on testosterone therapy immediately after the operation But if the testes are allowed to regress following hypophysectomy, testosterone alone will not restore spermatogenesis, and follicle stimulating hormone is required in addition The present studies suggest that this is because testosterone is only capable of maintaining the secretion of androgen-binding protein by fully developed Sertoli cells

If the male germ cells make inhibin which regulates follicle stimulating hormone secretion, and if this in turn controls the rate of spermatogenesis by influencing the secretion of androgen-binding protein from the Sertoli cells, we have a subtle biochemical interplay between the germinal and somatic elements of the testis But what of the ovary? The anatomical relationship between the cumulus cells and the oocyte is reminiscent of that between the Sertoli cells and the spermatogonia Does the mature oocyte produce the female equivalent of inhibin, and hence regulate gonadotrophin secretion? Do pituitary gonadotrophins act on the granulosa cells to influence secretions which in turn regulate maturation? Little thought has been given to such problems, and yet they might add a new dimension to our understanding of the complex sequence of endocrine events leading up to ovulation

What are the parents?

from W T Toner

NEW ideas are needed to explain muon to pion production ratios as large as 10⁻⁴ in high energy proton interactions Unlike pions, muons and electrons are not strongly interacting and should not be seen in these collisions, except as rare decay products of strongly interacting parents

Anomalously high production of large transverse momentum muons and electrons was reported last summer by several groups in the USA, at CERN and in the USSR, working with protons at energies above 70 GeV Roughly equal amounts of μ^+, μ^-, e^+ and e^- are observed In a recent issue of *Physical Review Letters* (**34**, 103, 1975) Leipuner *et al* claim a similar result for muons with no transverse momentum, in proton-nucleus collisions at only 28 GeV

It is ironic that this latest result comes from a re-analysis of a 1969 experiment which showed the absence of one hundred times larger amounts of 'direct' muon production, then claimed by a Utah group to take place in cosmic ray interactions Those claims found no support and were later withdrawn At that time, there was no compelling reason to make a painstaking analysis of the small residue, which was thought to come from the rare electromagnetic decays of the ρ^0, ω^0 and φ^0 vector mesons, strongly interacting analogues of the photon But models based on this idea require that most of the pions observed at the same energies and angles as the muons originate in the other decays of these same three particles the ρ^0, ω^0 and φ^0 have to be singled out from a hundred and one other ways of generating the ubiquitous pion

Gordon *et al* (*Phys Rev Lett*, **34**, 284, 1975) suggest that this may happen at large transverse momentum, if the rapid increase that they observe in the yield of ρ^0 relative to pions continues beyond 1 GeV/c transverse There is an intuitive appeal to such an idea, since the production of all types of particles at large transverse momentum is unexpectedly high and shows features suggestive

of some electromagnetic analogy How to turn it into a theory is another matter Besides, another mechanism still has to be found to explain Leipuner's data at low transverse momentum, where vector mesons can only account for 10% of the observed muon yield The decay of the η meson (a kind of heavy neutral pion) into two muons and a photon might account for another 15% in this case, but would upset the observed balance between electrons and muons if used to explain the large transverse momentum data

Unfortunately, lack of data on the production of unstable particles prevents us dismissing out of hand the unlikely possibility that known heavier relatives of the ρ^0, ω^0 and φ^0 give rise to the bulk of the muons and electrons They are included in the generic title 'heavy virtual photons' given to the unknown parents

An assortment of ordinary mechanisms would not satisfy the discoverers of direct muons, whose searches had a more glamorous stimulus Theorists have found it necessary to postulate the existence of many new particles in order to account for various features of the weak interaction, more than just the field particles needed to carry the weak force Those ideas have gained more support with the recent discovery of neutral weak currents

According to one class of theories, there should be a whole set of new strongly interacting particles with a new quantum number 'charm' There could even be a fourth degree of freedom, 'colour', to add to isotopic spin, strangeness and charm and give rise to its own family of particles Strong decays of the new particles would be inhibited by selection rules, giving plenty of opportunity for 'direct' muons and electrons to be produced by weak or electromagnetic decays

The J and ψ particles may fit such a scheme, but if their production is as infrequent as is rumoured they cannot

be the source of so many direct muons Are there others, with masses low enough to be produced in 28 GeV proton–uranium collisions?

We can expect many results bearing on these questions in the next few months A new impetus has been given to attempts to measure the production characteristics of the well known unstable particles The complete results of Ting's J-particle experiment should answer some of the mundane questions as well as the more exciting ones Every high energy laboratory is mounting programmes to search for charm and colour Also, many groups are looking again at their 'zoos' of supposed artefacts, conscious that neutral currents, J-particles and the direct muons all gave signs of their presence in earlier experiments, though too faint to create universal enthusiasm for following them up

Polyacrylamide gels

from Paul Calvert

CROSS-LINKED polyacrylamide gels are extensively used in the electrophoretic separation of proteins It is thought that the gel acts as a molecular sieve with a distribution of pore sizes in the 20 Å range so that small molecules pass easily through and large molecules are retarded These gels are prepared by the free radical polymerisation of an aqueous solution of a few per cent acrylamide containing a small amount of cross-linking agent Under these conditions one might reasonably expect to get a homogeneous network Pore sizes calculated on this assumption (see for instance Robard and Chrombach, *Proc natn Acad Sci USA*, **65**, 970, 1970, Hersey and Rees, *Nature phys Sci*, **230**, 92, 1971) are about right for protein dimensions and thus we have a fine example of a simple theory giving good results

Unfortunately for lovers of simple theories, recent scanning electron microscopy of freeze dried gels suggests that the structure is not homogeneous at all Blank and Reimschuessel (*J Materials Sci*, **9**, 1815, 1974) studied similar polyacrylamide gels from the point of view of their suitability as support media for crystal growth Their results show a structure consisting of 2–10 μm walls, surrounding pores of similar dimensions, in a 2 5 weight per cent acrylamide gel As the concentration is increased the walls become thicker This size is suitable for the growth of crystals but huge compared to any protein molecule so that if there is a sieving action it must be within the walls, and will depend on the pores not being interconnected

First we must consider whether the pores are an artefact of the preparation

technique The gels were prepared b ^{60}Co irradiation rather than by catalyst-accelerator system but thi should not be a crucial difference Th usual concentration of cross-linkin agent was used The freeze drying ma have distorted the structure but th kind of large-scale chain breakag needed to create the pores seems un likely Further, a cross-linked polyviny alcohol gel showed only very smal pores after freeze drying

There have been many reports o anomalous behaviour of organic gels In gelatin gels Johnson and Thorntoi (*J Photog Sci*, **16**, 117, 1968) showe that 1 μm particles have considerabl mobility Pines and Prins (*Macro molecules*, **6**, 888, 1973) have demon strated phase separation in agarose anc some polyvinyl alcohol gels but not ir gelatin

Thus there is some evidence for a macroscopic pore structure in severa gel systems which must arise by a liquid–liquid phase separation process analogous to that observed in many polymer solutions below the theta temperature (see Flory's *Principles of Polymer Chemistry*) but with the additional constraint of the cross-linked structure to be accounted for In terms of the electrophoretic separation the presence of the macroscopic pores cannot dc much good and it seem that further study of the relationship between structure and separation could lead to improved gel systems

Changes in species diversity

from Peter D Moore

IF there is one area of ecology in which theoretical speculation has outpaced acquisition of field data it is in the study of succession The time scale involved in most terrestrial successions is of the order of centuries, which leaves the hopeful student with limited options he must either be contented with the study of a series of extant phases which are assumed to be related in a successional series, or he must turn to systems which attain a more rapid equilibrium (such as plankton) for the formulation or confirmation of generalised models Neither course is satisfactory, but life is short

The former alternative has recently been chosen by Nicholson and Monk (*Ecology*, **55**, 1075, 1974) as a means of investigating the vexed problem of diversity changes during succession By obtaining data on the vascular plant composition of 51 seral vegetation types developed on abandoned agricultural land in the Georgia Piedmont, they have produced one of the most extensive sets of information currently avail-

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ble for the study of diversity changes

The mathematical definition of diversity is in itself a controversial subject Perhaps the most commonly used and widely accepted index of diversity is that derived from the information theory of Shannon and Weaver, which was first introduced into the ecological arena by Margalef (*Gen Syst*, **3**, 36, 958) Although this function has advantages as a general index, it has one major drawback, this being that it compounds species richness (number of species per unit area or in relation to the total number of individuals) and equitability or evenness (the apportionment of individuals among the species present) Both Odum (*Science*, **164**, 62, 1969) and Pielou (*An Introduction to Mathematical Ecology*, Wiley, New York) have pointed out that these two parameters may behave differently during succession and that such differences would be obscured by a simple consideration of the Shannon function

Nicholson and Monk have calculated richness, information content (Shannon diversity) and equitability for their data and in doing so have confirmed Odum's and Pielou's suspicions Richness and equitability (they use Pielou's index, *J*, which divides the Shannon index by the natural logarithm of the number of species see *J theor Biol*, **13**, 131, 966) do behave differently and information content follows an intermediate path Richness increases rapidly during the first 70 yr of abandonment and then continues to rise gradually over the next 130 yr, but within this general trend one can detect periods of stagnation at 10–20 yr (dominance of perennial grass species), 30–80 yr (pine dominance) and at 150–200 yr (climax species, such as *Fagus grandifolia,* attain dominance) Equitability, on the other hand, rises to fairly high levels within 20 yr and subsequently levels off, there is even a tendency to fall in the older stands, possibly due to the increasing degree of dominance

Falling diversity in the later stages of succession has also been observed by Loucks (*Am Zool*, **10**, 17, 1970) in the western Great Lakes region and by Shafi and Yarranton (*Ecology*, **54**, 897, 973) in the boreal forest of northern Ontario These authors interpret this as a product of random catastrophic disturbances which typify temperate and boreal vegetation types For example, within much of the boreal Canadian forests burning is likely to take place at intervals of 200 yr or less Under such conditions, evolutionary pressures might favour 'immature' vegetation types with low equitability in contrast to tropical forest where low perturbation frequency would favour high species richness and equitability

Nicholson and Monk favour the concept of evolutionary influences upon

ecosystem composition as far as richness is concerned, but they consider the equitability component of diversity to be more directly constrained by limited resource availability This would account for the early plateau in the equitability curve since, if resources were limiting, population and biomass growth within any stratum of the vegetation would be curtailed and hence equitability stabilised

From this work it is evident that diversity should not be considered as a single entity when dealing with successions, but should be reduced to its component parts It is sad that such analyses of diversity invariably, and perhaps inevitably, deal with single taxonomic groups of organisms such as birds, vascular plants, or lizards Maybe one should be consoled by the results of Murdoch, Evans and Peterson (*Ecology*, **53**, 819, 1972) who found positive correlation between evenness and diversity of plants and evenness and diversity of Homoptera within old field successions in Michigan Perhaps a consideration of plants alone does have some relevance and relationship to the behaviour of the entire biota during the course of succession, but over-generalisation could still be very misleading

Probing the transcript

from Paul Tolstoshev

THE complementary DNA copy of specific messenger RNA molecules has provided the molecular biologist with a powerful analytical tool Such cDNA molecules are synthesised from mRNA by viral RNA-dependent DNA polymerase, and can be labelled to very high specific radioactivities Molecular hybridisation analysis, using such a cDNA probe, allows the detection of extremely small amounts of a specific mRNA sequence

The analysis, with globin specific cDNA, of RNA transcribed from isolated chromatin by *Escherichia coli* RNA polymerase, has yielded spectacular results The *E coli* enzyme seems to transcribe globin-specific sequences from duck reticulocyte chromatin (Axel *et al*, *Proc natn Acad Sci U S A*, **70**, 2029, 1973), from mouse foetal liver chromatin (Gilmour and Paul, *Proc natn Acad Sci U S A*, **70**, 3440, 1973) and from rabbit bone marrow chromatin (Steggles *et al*, *Proc natn Acad Sci U S A*, **71**, 1219, 1973), but not from duck liver, rabbit liver, or mouse liver chromatin, or from free duck DNA Such results imply that isolated chromatin retains some specificity with respect to the genetic sequences which are available for transcription Furthermore, the

specificity of transcription seems to be mediated by the non-histone protein fraction of the chromatin This conclusion was derived from the chromatin reconstitution experiments of Paul *et al* (*Cold Spring Harb Symp quant Biol*, **38**, 885, 1973), and has recently been confirmed by the experiments of Barrett *et al* (*Proc natn Acad Sci U S A*, **71**, 5057, 1974)

A major problem in assessing the validity of chromatin transcription experiments of this type lies in the presence of endogenous RNA sequences in chromatin preparations Most chromatin preparations contain RNA, and it is perhaps to be expected that globin-specific sequences are contained in the RNA present in chromatin from erythropoietic tissue The cDNA probe cannot, of course, distinguish between endogenous and newly synthesised globin sequences Paul *et al* labelled the transcribed RNA with ^{32}P The hybrid between cDNA and transcribed RNA contained sufficient ^{32}P radioactivity to indicate that a large portion of the globin sequences detected had been newly synthesised But the very small amounts of ^{32}P label in the hybrid make quantitative estimations rather imprecise

Barrett *et al* measured amounts of endogenous globin sequences in RNA from native chicken reticulocyte chromatin, and found relatively high levels (about 0 02% of the total endogenous RNA) Transcription of native reticulocyte chromatin by *E coli* RNA polymerase yielded additional globin-specific sequences in amounts approximately the same as those of the endogenous sequences But when chromatin was separated into DNA, histone and non-histone components, and was reconstituted, either from chicken erythrocyte DNA and histones, and reticulocyte non-histone components, or from all reticulocyte components, no endogenous globin-specific sequences were detected Transcription of the reconstituted chromatins yielded RNA containing 0 007 to 0 025% globin-specific sequences Thus it seems that the preparative procedures used by these authors for DNA, histone, and non-histone protein isolation allowed few or none of the endogenous globin-specific sequences to survive This was confirmed, for the non-histone protein fraction, in a control experiment where erythrocyte DNA and reticulocyte non-histone protein were reconstituted in the absence of any histone When this material was transcribed, no globin-specific sequences were detected

This suggests that there are no globin sequences contaminating the reticulocyte non-histone protein fraction, as well as establishing a histone dependence for correct chromatin

reconstitution Barrett *et al* were able to demonstrate that substitution of the reticulocyte non-histone proteins by chicken liver non-histone proteins prevented the transcription of globin sequences The specificity observed in chromatin reconstitution must therefore be attributed to components of the non-histone protein fraction

The use of such chromatin reconstitution systems as a functional assay for specific non-histone proteins still however remains a formidable task This may be simplified somewhat if purified euchromatin fractions can be successfully reconstituted in the same way (Euchromatin is that fraction of the chromatin thought to be active in transcription *in vivo*) Several groups have isolated fractions of chromatin which seem to have many of the properties expected for euchromatin There has however been, to date, no demonstration that the DNA of such fractions is enriched for the gene sequences which are transcribed *in vivo*, in the cell of the chromatin's origin

An interesting study on the nature of the RNA transcribed *in vitro* from isolated chick myoblast chromatin, by a mammalian RNA polymerase (form B from calf thymus) has recently been reported by Groner *et al* (*Proc natn Acad Sci USA*, **72**, 194, 1975) These authors found that most of the transcribed RNA was of relatively small size (5 to 7S) They demonstrated, by isopycnic gradient centrifugation in CsCl, that a large proportion of this transcribed RNA remained bound to the DNA template The addition of a polyanion, heparin, to their reaction mixture resulted in an increase in the amount and size of the RNA transcript The RNA now sedimented as a broad peak (between 5 to 50S) on sucrose density gradients CsCl density gradient analysis revealed that the larger RNA had apparently been released from the template Pulse-chase experiments established that the small, DNA-bound transcript produced in the heparin was a precursor of the larger material made when heparin was added

A similar effect of heparin on the amount and size of RNA transcribed from chick oviduct chromatin by both mammalian and bacterial polymerase had previously been reported by Cox (*Eur J Biochem*, **39**, 49, 1973) In this case the effect was attributed to the inhibitory effect of heparin on nucleases present in the chromatin Control experiments by Groner *et al* showed that the chromatin used in their study was sufficiently free of ribonuclease activity to eliminate this factor as the major effect of heparin Groner *et al* interpret their results as indicating that the small RNA transcripts made in the absence of heparin are transcribed

from, and remain bound to single-stranded regions of DNA The addition of heparin induces a structural change in the template, resulting in the unwinding of longer stretches of DNA This, in turn, allows production of much longer transcripts, which are presumably displaced from the template by reformation of the DNA duplex As the authors point out, their interpretation is consistent with the model of chromatin structure where the unwinding of DNA to single-stranded regions is required for specific transcription (Crick, *Nature*, **234**, 25, 1971)

Two problems arise in this interpretation of the data First, heparin can cause displacement of chromosomal proteins from DNA (see for example Cox, *ibid*) and Groner *et al* did not determine whether such displacement was caused by the relatively high levels of heparin (2 5 mg ml^{-1}) used in their experiments Second, a search for single-stranded regions of DNA in chromatin by Levy and Simpson (*Nature new biol*, **241**, 139, 1973) revealed only very low levels (no more than 0 01% of the DNA) Nevertheless, it may be of interest to apply the approach of Groner *et al* to the transcription of reconstituted chromatin The use of specific cDNAs may make possible a demonstration that heparin (or other polyanions) can cause release of the transcriptional specificity of chromatin Thus one might perhaps obtain transcription of sequences not normally produced in the cells of the chromatin's origin Whether the action of heparin is mimicking, in some gross way, the action of specific gene regulators in eukaryote cells, remains to be established It is however clear that the *in vitro* reconstitution and transcription of chromatin will continue to be the focus of a great deal of attention in attempts to unravel the intricacies of transcriptional regulation

Pyroxene geotherm supports plumes

from Peter J Smith

In spite of their importance, temperatures in the Earth's interior remain uncertain because they are impossible to measure directly and difficult to estimate indirectly Recently, however, Boyd (*Geochim Cosmochim Acta*, **37**, 2533, 1973) applied pyroxene geothermometry to ultramafic xenoliths in the kimberlite pipes of Lesotho and derived a temperature–depth curve which could well represent real temperatures in the upper mantle He used phase equilibria in the enstatite–diopside and enstatite–garnet systems to obtain the equilibration temperature and pressure of xeno-

liths containing the mineral assembla; enstatite+diopside+garnet The resul ing geotherm suggests that down about 160 km temperature increas with depth in reasonable accord wii the extrapolation of the near-surfac thermal gradient But at a depth (about 170 km the temperature indicate by the pyroxene geotherm begins to ri much more rapidly, increasing t several hundred degrees over a dept of a few tens of kilometres

It is difficult to imagine that th rapid temperature rise represents stead state behaviour But if it is transier (with respect to the lithosphere), wh; causes it? Boyd's own suggestion w; shear heating, which would also explai why the xenoliths from below 170 kr are strongly sheared whereas thos from above are unsheared Unfoi tunately, this hypothesis fails to explai why the intrusions carrying the xenc liths are localised phenomena second possibility would be therm; conductivity variation, but the therm; conductivity would have to decrease b a factor of five, a possible but unlikel change

Parmentier and Turcotte (*Eart, planet Sci Lett*, **24**, 209, 1975) there fore propose that the increase in ge(thermal gradient reflects a relativel localised distortion of the norm; mantle convection pattern caused by mantle plume As they envisage it, col(lithosphere moves over a hot risin; plume The extra heat arriving at th base of the lithosphere would normall; be expected to rise up through th lithosphere by conduction But conduc tion is slow, so that before the hea has penetrated very far, any particula section of lithosphere will have mover away from the plume zone The geo therm in the lithosphere will thu hardly be affected by the plume, excep near the base In other words, the litho spheric and asthenospheric geotherms which are different, remain largely in dependent of each other, and thi accounts for the point of inflection a about 170 km (although the smal amount of conduction into the litho sphere ensures that the two geotherm merge smoothly) By the same token the depth at which the temperature begins to rise rapidly is the lithosphere- asthenosphere boundary

Numerical studies of a model involv ing a cylindrical plume heated from below give results which are consisten with this view That is to say, the total geotherm predicted from the model i in good agreement with Boyd's pyrox ene geotherm as long as the kimberlit(intrusion in the model is located awa) from the plume axis in the directior from which the lithosphere is approach ing Clearly this agreement may b(taken as indirect evidence in favour o| the existence of mantle plumes

articles

Mesozoic apparent polar wander and Atlantic plate tectonics

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New palaeomagnetic data indicate that the North American apparent polar wander curve followed an approximately latitudinal path for most of the Mesozoic This has important implications concerning the early phases of seafloor spreading in the Atlantic, particularly as Africa seems to have been stationary at that time

THE North American apparent polar wander (APW) curve for the mid Mesozoic is poorly understood and has been the subject of disagreement for some time Data have been particularly inconsistent for the Jurassic Period[1,2] With relatively few reliable Jurassic determinations it has commonly been thought that the APW curve passed from a reasonably well determined point in the Triassic (see Fig a) to a position which is close to the present axial dipole, and then to a well determined Cretaceous position In fact, the Mesozoic APW curve does not pass through the present axial dipole, but follows an approximately latitudinal path present-day coordinates) between the Triassic and Cretaceous pole positions[3,16]

The direction of the path of the pole during the Mesozoic has a direct bearing on our understanding of the early

opening of the Atlantic, especially in light of African palaeomagnetic data Published African palaeopoles for the early Triassic to the early Cretaceous are so similar in their positions as to be indistinguishable from one another at the level of statistical confidence of palaeomagnetic data (Fig 1b) This consistency indicates that Africa was essentially fixed relative to the magnetic pole for most of the Mesozoic The apparent motion of the North American pole, therefore, indicates that the entire motion related to the early opening of the North Atlantic (the separation of Africa from North America) must have been taken up by movement of the North American plate

Before examining the constraints that the newly established North American Mesozoic APW curve places on the opening of the Atlantic, a few points regarding the curve itself need to be examined

North American Triassic poles used by various workers in the reconstruction of the Atlantic always include a group of late Triassic poles displaced more to the north than the rest of the late Triassic poles They have been cited[4] as evidence of rapid polar wander Obviously, the tight grouping of the African poles refutes this hypothesis

Poles located at these higher latitudes are all derived from
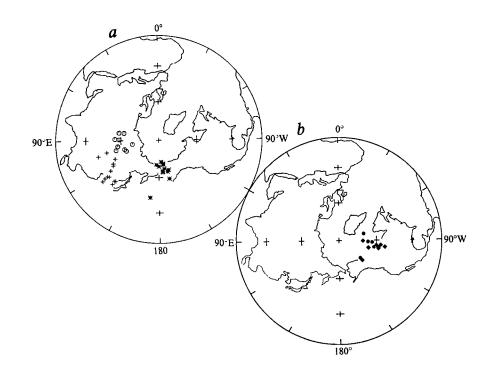
Fig. 1 *a*, Most reliable[16] published poles for the Permian (+), Triassic (O) and Cretaceous (∗), making up the APW curve for North America *b*, Most reliable poles (see refs 9 and 15) for the Triassic (●), Jurassic (◆) and Cretaceous (∗) of Africa
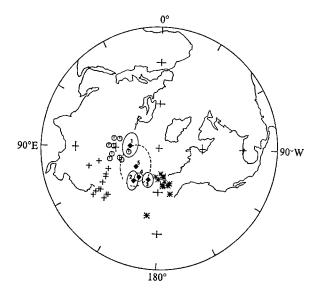
Fig. 2 The North American, Mesozoic APW curve Poles (♦) and ovals of confidence of 1, the Summerville Formation[1], 2 and 3, lower and upper Morrison Formation[16], 4, Mesozoic dykes[6], 5, Topley Intrusions[3], against the background of the Permian (crosses), Triassic (open circles) and Cretaceous (*) poles of Fig 1*a*

rocks of the north-eastern USA and have not been observed in late Triassic rocks of western North America These poles must be viewed with suspicion as they form a streak between the majority of late Triassic poles and the present axial dipole Armstrong and Besancon[5] contend that the Newark Group rocks have been somewhat altered (zeolite facies metamorphism) and are unreliable for K–Ar dating, as most magnetic studies are from rocks associated with the Newark Group it is therefore possible that some of the magnetic directions obtained are unreliable Regardless of the reason, the likelihood that a real streak would occur in the same direction as that expected from the imprinting of secondary magnetisation is so improbable that it should be questioned Furthermore, a number of the studies have a very small sample population

Previous studies on Jurassic rocks have given very inconsistent results The position of the pole determined from the Upper Jurassic Summerville Formation is, however, not much different from that of Triassic time[1] There are no structural uncertainties associated with the data and remagnetisation of a younger unit into an older direction is impossible It is unlikely that the data record an odd direction resulting from secular variation as they encompass a reversal of the field, and therefore probably span enough time to average out extremes of secular variation The Oxfordian Summerville direction seems to indicate that the pole position for North America remained relatively constant from late Triassic to late Jurassic time and that it subsequently moved rather quickly to the Cretaceous position

Magnetic directions obtained from the unit directly overlying the Summerville, the Morrison Formation[16] are in complete accord with the hypothesis that most Mesozoic APW relative to North America occurred over a relatively short time during the late Jurassic Two different directions have been obtained from sediments in the lower and upper parts of the Morrison formation (Kimmeridgian–Tithonian respectively The directions lies in sequential position on the APW curve and suggest that about 10° of apparent motion of the pole occurred during Morrison time These directions also imply that about twice that amount of apparent motion occurred between the Summerville and the lowest sampled portion of the Morrison (Fig 2)

(More than 35 m of unsampled sediments, and a probab unconformity, exist between the lowest sampled beds the Morrison and the Summerville) The Morriso and Summerville data indicate that the majority of th movement occurred in post-Summerville time, that is, lat Oxfordian(?), Kimmeridgian and Tithonian The olde dated Cretaceous rocks studied palaeomagnetically sho pole positions already located in the position shown by lat Cretaceous rocks

The Morrison data also confirm that the APW path w approximately latitudinal relative to the present axial po and did not include the present dipole The averag direction of the Topley Intrusions[3] agrees with this hyp thesis, although the confidence limits on the data are qui large A pole in the same area has also been obtained t DeBoer[6] from some dykes of unknown age cutting Upp Triassic rocks

This APW path requires that the pattern of separation Africa and North America be somewhat different from th proposed previously Pitman and Talwani[7] have compute finite difference poles to describe the rotations needed t open the central Atlantic Rotation of the North America plate about the oldest (180–155 Myr) pole of opening w produce very little apparent motion of the magnetic pol relative to North America as the magnetic and rotation poles are very close together That supports my finding that the magnetic pole was approximately static relative t North America from the late Triassic through the m Jurassic and into the beginning of late Jurassic time

Rotation about Pitman and Talwani's[7] next pole c opening (155–81 Myr ago), however, does not move averag late Triassic poles[8,9] through the positions represented b the Morrison poles Nor does this rotation move the pole t the position of the North American Cretaceous poles Th positions of the lower Morrison, Topley, and perhaps th Mesozoic dyke poles suggest a two stage rotation during th Jurassic one additional rotation to that suggested by Pitma and Talwani[7] for the time between rifting and 81 Myr BP The pole to this rotation must lie on a great circle tha passes midway between the late Triassic and lower Morriso poles The rotational pole must lie at some point betwee the small circle connecting these magnetic poles and a poin on the North American continent, in order to produce bot the APW observed and the actual motion of North Americ relative to Africa This indicates that North America mus have undergone a clockwise rotation relative to Afric before it moved away causing the opening of the Atlantic The rotation following the clockwise rotation was probabl about Pitman and Talwani's[7] 180–155 Myr pole of opening as that moves the pole approximately from the lowe Morrison position through the upper Morrison position an into the Cretaceous position

The path of polar motion defined by the sequentia Summerville and Morrison poles corresponds to a change ir direction of APW during the Jurassic (Fig 2) The timing of this change of direction is interesting The Morrison polarity sequence can be correlated[16] with the oldest anomalies of an M sequence anomaly pattern[17] Thus, the lower Morrison pole represents the pole of the oldest of the Mesozoic anomalies This means that the change in direction of the pole path occurred just before the formation of the lineated anomalies The change coincides with the changes from rough to smooth and from magnetically quiet to lineated-anomaly sea floor, and may, therefore, indicate a causal relationship

The stationary position of Africa throughout most of the Mesozoic may have been conducive to asymmetrical spreading in the Atlantic Indeed, the quiet zone boundaries on either side of the Atlantic are not symmetrical with respect to the continental margins[10,11] At least two mechanisms could produce these different widths of sea floor on either side of the Mid-Atlantic Ridge[12] either an eastward jump

of the ridge crest or a migrating ridge crest, would produce asymmetry. The stationary position of the African plate requires that the spreading crest be in motion at the time of formation of the marginal quiet zone and the late Jurassic–early Cretaceous anomalies. These data therefore favour the migrating ridge system as the cause of the asymmetry, suggesting that both the North American plate and the ridge crest may have been migrating away from Africa, with the plate moving much faster. Later, however, during the time between the end of the quiet zone and anomaly 32, the African pole still shows no motion although the zones of the sea floor representing this time are quite similar in width on both sides of the Atlantic. This suggests that the ridge crest may by then have started to migrate rapidly enough to produce symmetrical spreading during that time.

If the Morrison and Summerville poles are truly representative of sequential positions of the Jurassic pole along an APW path created by seafloor spreading, very high rates of seafloor motion are implied. An approximate idea of the rates involved can be obtained from equations used by Deutch[13]. Although Pitman and Talwani's[7] rotational pole does not rotate the mean late Triassic pole into the position of the mean Cretaceous pole, an approximate seafloor spreading rate can be obtained by computing the rate of plate movement about this rotational pole that would bring the Triassic pole closest to the Cretaceous pole. The amount of time allowable for the rotation is considered to be approximately 20 Myr (early Oxfordian to early Cretaceous —for numerical ages see ref. 14), based on the position of the Summerville pole. I have computed the rates at 45° away from the rotation pole, that is, within the area of the sea floor between Africa and North America. A rate of 16–19 cm yr⁻¹ is necessary at this distance to produce the total opening for that time[7]. (The uncertainty stems from the choice of the various[1,8,9] Triassic poles which can be used.) The rate for just the motion between early Morrison and early Cretaceous time (around this same rotational pole), allowing 15 Myr (from the base of the Kimmeridgian to the base of the Cretaceous), at 45° from the pole, would be 12.5 cm yr⁻¹. These are quoted as whole rates, as the ridge system was probably also in motion. They are rates for the time of formation of some portion of the quiet zone and anomalies M22 to M25.

Even if the rates are considered in terms of half rates, both computations give a surprisingly high rate of seafloor spreading. The rates are far in excess of any that have been proposed for the Atlantic, and rank among the highest rates ever proposed for any spreading ridge. Such high rates may have occurred as some consequence of the initial spreading process, or they may indicate events that caused a drastic slow-down in Atlantic spreading subsequent to opening.

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Nuclear envelope permeability

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The permeability of the amphibian oocyte nuclear envelope in situ has been determined for three tritiated dextrans. The envelope is a sieve, restricting molecular movement between the cytoplasm and nucleus. The patent radius of its pores is about 45 Å.

MANY substances are known to be in continuous exchange across the nuclear envelope. These include ions and metabolites[1-3]; histone and non-histone chromosomal proteins[4-6]; ribosomal and messenger ribonucleoproteins[7-9]; hormones, either free or bound to protein receptors[10]; viral proteins and nucleic acids[11-13]; and less characterised protein and RNA species[14-15]. Yet, because quantitative flux data are almost non-existent, we know little about the factors that govern the passage of these substances through the nuclear envelope, and we cannot assess the envelope's role in controlling nucleocytoplasmic exchange.

The most direct method of obtaining rate data on nuclear envelope permeation is to microinject a substance into the cytoplasm and follow its passage into the nucleus with quantitative optical or electron microscopic techniques. Solutes used for such studies have been chosen primarily for technical rather than heuristic reasons. With the advent of ultra-low temperature autoradiography (ULTA)[16], which can quantitatively locate any tracer substance regardless of its diffusibility, a systematic approach to the problem of envelope permeation becomes possible, since any radioactively labelled substance can serve as a probe.

Size has been the most studied of the permeant properties which influence nuclear envelope passage. Colloidal gold particles greater than 125–145 Å in diameter do not penetrate the nuclear envelope of Amoeba proteus[17]. Ferritin, with a molecular diameter of 95 Å, does not enter the nuclei of amphibian and cockroach oocytes[18,19]; below this limit, permeation is inversely related to tracer size. Passage of proteins (molecular weight 12,000–67,000; radii ≈ 15–35 Å) is slowed by the nuclear envelopes of the cockroach oocyte[19] and chironomid salivary gland cell (P.L.P., unpublished), the degree of slowing increasing with increased molecular size. Slowing is not observed when tracers are 4,200 dalton or less.

While these observations reveal some nuclear envelope sieving properties, a more quantitative understanding requires nucleocytoplasmic flux and equilibrium data on a size-graded series of molecules with otherwise similar properties. We have obtained such data for size-characterised fractions of dextran, a polymer of D-glucopyranose units linked by α-1:6, α-1:4, and α-1:3 glucosidic bonds. Dextrans in aqueous solution are considerably hydrated and behave as spheres[20]. The hydrodynamic radii of our tracer fractions were 12.0, 23.3 and 35.5 Å (Table 1). Solutions of purified ³H-dextran were microinjected into the cytoplasm of mature oocytes of Rana pipiens. Solute diffusion was permitted until the cells were quenched at −190 °C. The oocytes were sectioned at −50 °C, and the local intracellular concentrations of tracer determined using ultra-low temperature autoradiography. Figures 1 and 2a and b summarise the preparation and microinjection of the ³H-dextran fractions, the autoradiographic procedures, and the tests for intracellular ³H-dextran degradation.
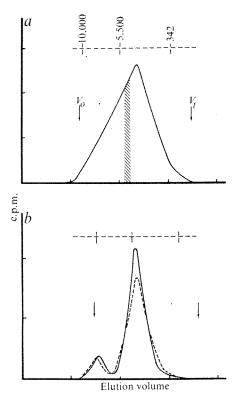
Fig. 1 *a*, Gel chromatographic isolation of 12.0 Å ³H-dextran tracer. Commercially obtained ³H-dextran (specific activity 0.4 mCi mg⁻¹, lot No. 622-215, New England Nuclear, Boston) in water was fractionated on Sephadex G-50 to remove > 10,000 dalton dextrans (not shown). A portion of the remaining material was rechromatographed on Sephadex G-50, yielding the distribution shown. A subfraction (shaded), with hydrodynamic radius 12.0 Å, was isolated for injection and was concentrated by lyophilisation to 650 µCi ml⁻¹. *b*, The size distribution of the injectate was confirmed on Sephadex G-50 at the time of microinjection (————); no breakdown and only negligible polymerisation (a time-dependent process observed with 12.0 Å dextrans) was detected. Intracellular stability was established by homogenising four cells 40 h after microinjection and chromatographing the 12,000*g* supernatant (. . . . .). ³H-dextran injectates (23.3 and 35.5 Å) were prepared similarly using Sephadex G-50 and G-75 columns. All columns were equilibrated with water and eluted to stable total volumes (*V*ₜ) before use. Void volumes (*V*₀) were determined with Blue Dextran 2,000, and molecular size calibrations were carried out using sucrose (342), inulin (5,500) and dextrans (10,000, 20,000, 40,000 and 70,000) as standards.

Cytoplasmic diffusion
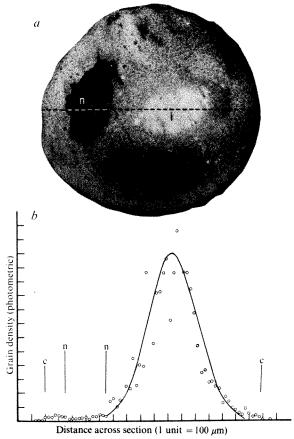
Immediately after microinjection, the ³H-dextran is found at the injection site, observed in autoradiographs as an intense concentration of silver grains. The tracer then diffuses radially from the injection site, and grain density transects show time-dependent diffusion profiles. Cytoplasmic diffusion coefficients, *D*c, are estimated by comparing the cytoplasmic profile at a known diffusion time (*t*d) with theoretical diffusion profiles (Fig. 2c). The analysis has been outlined previously for sucrose²⁶ and inulin²⁷ in the oocyte, for which *D*c are 2 × 10⁻⁶ and 3 × 10⁻⁷ cm² s⁻¹, respectively. Our estimates for *D*c of 12.0, 23.3, and 35.5 Å ³H-dextrans are 3.5 × 10⁻⁷, 2.5 × 10⁻⁷ and 1.5 × 10⁻⁷ cm² s⁻¹, respectively. Over the 70-fold molecular weight range of substances studied in the oocyte, *D*c are in the range 0.1*D*w – 0.4*D*w (*D*w is the solute diffusion coefficient in pure water); this agrees with estimates of *D*c/*D*w reported for a variety of tracers in other cells²⁶,²⁸.

Nuclear envelope permeation

Grain density profiles of oocytes in which the diffusion front has reached the nucleus reveal the nuclear envelope's influence on solute movement. Figure 3a–d, showing profiles for 12.0 Å ³H-dextran at *t*d = 3, 5 and 16 min, and 16 h, are transects made close to the injection site and through the nucleus.

At diffusion equilibrium, 12.0 Å ³H-dextran is uniformly distributed within both the nucleus and the cytoplasm (Fig. 3*d*), but it is more concentrated in the nucleus (see also Fig. 4*a*). A higher concentration in the nucleus than in the cytoplasm has been observed with every solute we have studied in the oocyte. This phenomenon has also been found in other cells and has been attributed to solute exclusion by the cytoplasm, a process widely associated with macromolecular gels²⁷.

At shorter diffusion times, 12.0 Å ³H-dextran gradients are discontinuous, decreasing sharply at the level of the nuclear envelope (Fig. 3a–b). Since this is obviously not caused by low nuclear solubility, the nuclear envelope must be a substantially greater transport barrier than is a similar thickness of cytoplasm. Intranuclear 12.0 Å gradients mirror those in the adjacent cytoplasm. The envelope, although a sufficient barrier to cause gradient discontinuities, is insufficient to isolate nuclear from cytoplasmic gradients. This suggests that the time required for 12.0 Å ³H-dextran to cross the nuclear envelope is less than that required to diffuse the radial distance of the nucleus.

Fig. 2 Microinjection was directed into the vegetal cytoplasm to eliminate the possibility of nuclear damage. Injectate concentrations were 1.6, 1.0 and 0.7 mg ml⁻¹ for 12.0, 23.3 and 35.5 Å ³H-dextrans, respectively; and volumes were 5 × 10⁻⁵ ml, or about 2% that of the cell. After a period of time (*t*d), the oocyte was rapidly embedded in OCT and quenched to – 190 °C in liquid nitrogen. Sectioning and autoradiographic exposure were carried out at – 50 °C and – 96 °C, respectively. This procedure prevents solute redistribution from the time of freezing until completion of the autoradiograph¹⁶. *a*, Autoradiograph, in darkfield illumination, of a section through both the injection site (i) and nucleus (n) of an oocyte injected with 12.0 Å ³H-dextran (*t*d = 8 min). Grain counting was carried out visually, in phase contrast at × 2,000, or photometrically, with a Leitz MPV-1 microscope photometer system employing Ploem incident-light illumination²⁴. Grain density values throughout have been corrected for the difference in nuclear and cytoplasmic water content²⁵ and are proportional to ³H-dextran concentrations on a water basis. *b*, Grain density profile taken along the axis shown as a dotted line in (*a*). The vertical bars, c and n, mark the cell and nuclear boundaries. The solid line is the theoretical diffusion gradient for *D*c = 3.5 × 10⁻⁷ cm² s⁻¹, assuming that the injection volume is small enough to constitute an instantaneous point source, and that solute reflection from the cell boundaries is negligible.
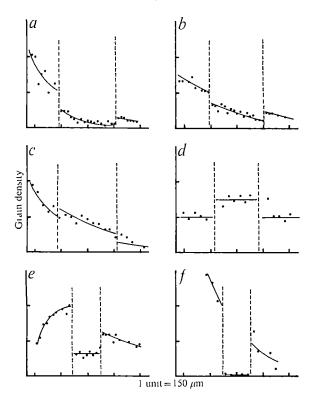
Fig 3 Grain density profiles through the nucleus (indicated by vertical dashed lines) and adjacent cytoplasm of ^{3}H-dextran injected oocytes a–d, 12 0 Å, $t_d = 3$ min, 5 min, 16 min, and 16 h, respectively, e, 23 3 Å, $t_d = 10$ min, f, 35 5 Å, $t_d = 42$ min

Figure 3e and f are grain density profiles for 23 3 and 35 5 Å ^{3}H-dextrans Both profiles decrease more sharply at the nuclear envelope than do the 12 0 Å ^{3}H-dextran profiles at comparable times, but, unlike the latter, the 23 3 and 35 5 Å profiles show uniform transnuclear concentrations, in spite of marked gradients in the adjacent cytoplasm These profiles demonstrate that the nuclear envelope is less permeable to larger dextrans than to smaller, and that envelope permeability can play a major role in limiting the rate of nuclear entry

Quantitative expression of the envelope's influence on solute transport is complicated by the fact that cytoplasmic and nucleoplasmic diffusions are not instantaneous, hence, each point on the nuclear envelope experiences different and changing concentrations of the permeating solute To deal with this problem, we compared solute concentrations in adjacent nuclear and cytoplasmic positions by determining the grain density, G, at Q positions $(i, j, k\quad)$ inside, but close to the edge of, the nucleus $(G_n^i, G_n^j\quad)$ and in the adjacent cytoplasm $(G_c^i, G_c^j\quad)$ The mean of the ratios of adjacent nuclear and cytoplasmic sites is the nuclear cytoplasmic grain density, given by

$$X_{n/c} = (G_n^i/G_c^i + G_n^j/G_c^j + \quad)/Q \tag{1}$$

$X_{n/c}$, expressed as a function of t_d, is a measure of the time course of nuclear envelope permeation Figure 4a shows this for the three ^{3}H-dextrans investigated The nuclear entry rates are markedly different, equilibrium is reached for 12 0 and 23 3 Å ^{3}H-dextrans in about 0 5 and 15 h, respectively, with an equilibrium distribution ratio, $K_{n/c}$, of 1 45 The 35 5 Å ^{3}H-dextran enters much more slowly, $X_{n/c}$ at 23 h being 0 1, and does not achieve equilibrium during the experiments

If cytoplasmic and nuclear diffusion are rapid as compared with envelope permeation, and if permeant dwell time within the envelope is short relative to t_d (as the profiles of Fig 3 suggest), the kinetics of diffusional nuclear filling can be described by

$$1 - (X_{n/c}/K_{n/c}) = \exp(-k_n t_d) \tag{2}$$

in which the rate constant, k_n, is given by

$$k_n = P_n A/V_n \tag{3}$$

where P_n is the nuclear envelope permeability coefficient, A is the envelope area available for permeation, and V_n is the nuclear volume If cytoplasmic or nucleoplasmic diffusion significantly affected nuclear entry, a higher order equation would be necessary to describe the kinetics The greater such intracompartmental diffusion influences, the more poorly equation (2) will describe nucleocytoplasmic tracer movement[28]
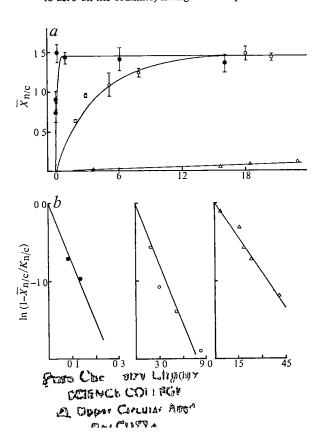
The nuclear entry kinetics of all three ^{3}H-dextrans are described by first order exponential equations of the form of equation (2), (Fig 4b–d) We conclude that $X_{n/c}$ corrects for spatial differences in tracer concentration arising from the transcellular diffusion, and that nuclear entry is primarily restricted by the nuclear envelope The experimentally determined rate constants, k_n, are given in Table 1 Although the 35 5 Å ^{3}H-dextran differs in molecular radius from the 12 0 Å by a factor of only 2 9 its rate of permeation differs by a factor of more than 2,500 This sieving effect seems to arise from the specialised porous structure of the nuclear envelope

Functional pore size

If we view the nuclear envelope as a uniformly permeable barrier, A in equation (3) is the total nuclear surface area and, assuming a spherical nucleus of radius r_n, we have

$$P_n = k_n r_n/3 \tag{4}$$

Fig. 4 a, Time course of nuclear envelope permeation, expressed as the average nuclear cytoplasmic grain density, $\bar{X}_{n/c}$ (see text), as a function of diffusion time after microinjection, t_d, for 12 0 (●), 23 3 (○), and 35 5 Å (△) ^{3}H-dextrans Vertical bars indicate s e mean b–d, First order exponential entry kinetics illustrated by plots of ln $(1 - \bar{X}_{n/c}/K_{n/c})$ as a function of t_d, for 12 0, 23 3 and 35 5 Å ^{3}H-dextrans, respectively $K_{n/c}$ for 35 5 Å ^{3}H-dextran is assumed to be 1 45, the same value observed for 12 0 and 23 3 Å ^{3}H-dextrans Note the different time scales on the abscissae The rate constants, k_n of Table 1 are given by the slopes of the linear least squares regression lines (constrained to zero on the ordinate) fitting the data points

Table 1 ^{3}H-dextran tracer data			
Radius (Å)	k_n‡ (s⁻¹)	P_n (cm s⁻¹)	A_p (cm²)
12 0*	$(2\,13 \pm 2\,94) \times 10^{-3}$	$1\,60 \times 10^{-5}$	$2\,21 \times 10^{-13}$
23 3†	$(7\,16 \pm 2\,61) \times 10^{-5}$	$5\,37 \times 10^{-7}$	$1\,04 \times 10^{-14}$
35 5†	$(8\,31 \pm 1\,66) \times 10^{-7}$	$6\,23 \times 10^{-9}$	$2\,01 \times 10^{-16}$

*Determined from position of elution peak from Sephadex G-50 ($K_{av} = 0\,40$) and the calculations of Laurent and Killander[21]

†Weight average molecular weights (M_w) were determined by Sephadex chromatography with standard dextrans, and the corresponding molecular radii taken from the measurements of Granath and Kvist[22]

‡The k_n is the slope of the least squares regression line calculated to fit the data points and constrained to zero on the ordinate (Fig 4b–d) The ± value given is the 95% confidence interval for the slope[23], the confidence interval for 12 0 Å dextran is misleadingly large since only two pre-equilibration data points were obtained, and the method of calculation does not admit use of the relevant early equilibrium points shown in Fig 4a

P_n and A_p calculated as explained in text ($r_n = 225$ μm)

from which we can calculate the envelope permeability coefficient, P_n, for each ^{3}H-dextran (Table 1) P_n is probably not, however, a functionally significant measure of nuclear membrane properties There is reason to believe, from both envelope ultrastructure and electron microscopic studies of colloidal gold and in vivo ribonucleoproteins, that passage of macromolecules through the envelope is restricted to a cylindrical central channel of each nuclear pore complex[3] If this is true for our ^{3}H-dextran tracers, we can calculate the total effective envelope pore area available for permeation, A_p^{total}, substituting D_p/d for P_n in equation (3) where D_p is the ^{3}H-dextran diffusion coefficient within the pores, and d is the pore complex length Then

$$A_p^{total} = k_n V_n d / D_p \qquad (5)$$

and the mean effective area available for diffusion through each pore, A_p, is given by

$$A_p = A_p^{total}/N \qquad (6)$$

where N is the number of patent pores per nucleus The best available estimate of d is 1,500 Å (refs 29–33), and of N, $1\,97 \times 10^7$ (refs 34 and 35) D_p is assumed to be equal to D_c, and A_p for each ^{3}H-dextran is shown in Table 1

For a membrane with pores of radius r, A_p is equal to the total patent area of a pore, πr^2, reduced by two solute–pore interaction factors, each a function of a/r (refs 36 and 37) The first expresses the steric hindrance to pore entry[38], the second is a 'wall correction factor', K_1, describing the frictional resistance to diffusion within a cylindrical pore relative to that in free solution Thus, A_p decreases with increasing tracer radius a

$$A_p = \pi r^2 [1 - (a/r)]^2 (1/K_1) = \pi (r - a)^2/K_1 \qquad (7)$$

Haberman and Sayre[39] provided a theoretical solution for K_1 which fits well the available experimental results over a large range of a/r (ref 40) We have applied computer techniques to the solution method of Haberman and Sayre to calculate the values of K_1 over the range $0 < a/r < 1\,0$ at intervals of $0\,02$ units (P L P, and P Scherr, unpublished) The calculated K_1 values were used to construct the curves in Fig 5, which illustrates the dependence of A_p on a and r The experimentally determined A_p for 12 0, 23 3, and 35 5 Å dextrans correspond to r values, determined from equation (7), of 49 0, 40 7, and 43 3 Å, respectively The mean of these determinations is 44 3 Å, in Fig 5, the three plotted A_p values are best fit by the $r = 45$ Å curve We conclude that the ^{3}H-dextran nuclear entry kinetics can be explained quantitatively as restricted diffusion through nuclear pores with patent radii of about 45 Å

There is some uncertainty over the factors (V_n, d, D_p, and N) which enter the calculations of A_p We have determined the ranges
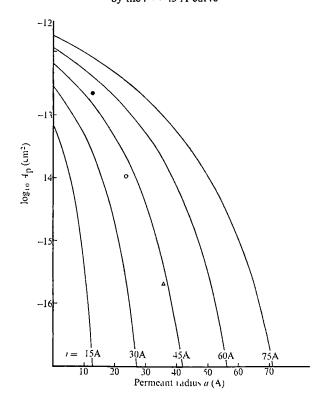
of A_p, and the corresponding ranges of r, resulting from reasonable ranges of each factor around the values used above For the 35 5 Å ^{3}H-dextran data, an error of ± 10% in the value of the nuclear radius (r_n) would correspond to a range of less than ±1 Å about the determined r Possible ranges for the value of D_p (0 1–0 4 × D_w), d (750–2,000 Å), (refs 30, 35 and 41), an N (1 02–3 12 × 10⁷), (refs 34, 35 and 42) each correspond ranges less than ± 3 Å about the 35 5 Å ^{3}H-dextran determine value of r Similarly, variation of ±10% in the value used fo 35 5 Å ^{3}H-dextran radius (a), results in a ± 4 Å change in r Thu unless all values chosen err in the same direction, determinatic of r from A_p and a by equation (7) is not greatly affected b possible errors in the values of the factors used

Permeability of the mature oocyte nuclear envelope

Results of earlier studies of the permeability of the matur amphibian oocyte nuclear envelope are consistent with th nuclear entry kinetics expected for passage through an envelop with pores of radius 45 Å, as reported here The solutes used, order of increasing size, include sodium[1], glycerol[43], cy teamine[44], sucrose[26], inulin[27], histones[18], polyvinylpyrrolidon coated colloidal gold[45,46], bovine serum albumin (BSA), (ref 18 and ferritin[18] Solutes the size of sucrose ($a = 4\,4$ Å, ref 36) o smaller reportedly enter the oocyte nucleus rapidly Intermedia size solutes—histones, colloidal gold, and BSA—enter mor slowly, while ferritin ($a = 47$ Å) does not enter at all

In principle, diffusion of all solutes is influenced by the nuclea envelope Our ability to detect this influence, however, is limited to cases in which half-times for nucleocytoplasm equilibrium, $t_{1/2}$, are at least several minutes—the time re quired to microinject and freeze the oocyte In practice th condition is met only with larger solutes, as illustrated in Fig 6 where $t_{1/2}$ for the mature oocyte nucleus at 15 °C is plotted as function of permeant radius, a As examples, in the oocyte wil

Fig. 5 Mean effective cross-sectional area for diffusion through each pore (A_p), as defined by equation (7), plotted as a function of tracer radius, a, for several pore radii, r Values of A_p determined experimentally (equations (5) and (6)) for 12 0 (●), 23 3 (○) and 35 5Å (△) ^{3}H-dextrans are indicated by points, and are best fit by the $r = 45$ Å curve
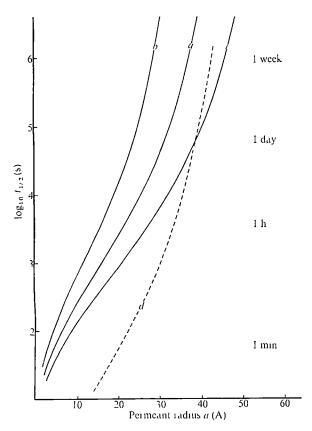
Fig 6 Half-times for nucleocytoplasmic equilibration of permeants with radius a by restricted diffusion through nuclear envelope pores (at 15 °C) a, Mature amphibian oocyte nucleus, with $r = 45$ Å, b and c, oocyte nucleus, if $r = 35$ and 55 Å, respectively, d, cell with 5 μm nuclear radius and $r = 45$ Å

$= 45$ Å a solute with radius 5 Å exchanges with a $t_{1/2}$ of bout 1 min, and the reported[27] inability to detect an envelope influence on oocyte nuclear entry of inulin ($a = 14\,8$ Å, ref 22, $_{1/2} \approx 10$ min) probably results from the use of too large a value or t_d as the earliest time studied

Since $t_{1/2}$ is proportional to nuclear radius (equations (2) and 3)), the entry half-time for a given permeant would be shorter n smaller cells than in the oocyte Fig 6d is a plot of $t_{1/2}$ against t for a 5μm radius nucleus assumed to share all of the properties other than size) of the oocyte nucleus The plot provides a notion of the time scale of nuclear permeation to be expected in he typically smaller cells of most multicellular organism tissues

Nuclear envelope permeability in other eukaryotes

Generalisations based on the present analysis will be valid only to the extent that the amphibian oocyte nuclear envelope is similar to other eukaryote envelopes Nuclear envelope ultrastructure is extraordinarily conservative, envelope similarity has been demonstrated in cells as phylogenetically distant as amphibian oocytes, amoebae, dipteran salivary gland cells, HeLa cells, normal and cancerous liver cells, and onion root tip cells[3,33,47] Several studies using insect oocytes[19,48], amoebae[49], HeLa cells[6], and salivary gland cells (P L P , unpublished), have yielded semi-quantitative nucleocytoplasmic exchange kinetics consistent with a patent pore radius about that determined in the present quantitative experiment These studies included microinjection of flourescein-labelled proteins, electron microscopic localisation of colloidal gold and ferritin, and autoradiographic localisation of endogeneous proteins Thus, both morphological and tracer studies suggest that the mature amphibian oocyte nuclear envelope is representative of eukaryote envelopes in general

The eukaryotic envelope consists of two 70–80 Å unit membranes, separated by a perinuclear space, and fused at intervals to form pore complexes 400–1,000 Å in diameter An element of each complex is a cylindrical proteinaceous structure extending through the complex into both cytoplasm and nucleoplasm[3] These structures are annular in cross section and are widely considered to be tubes with patent central channels of 50–300 Å, most frequently 100–150 Å (refs 32, 34 and 50) Three lines of evidence support the view that these pore-complex channels are the sites of nucleocytoplasmic macromolecular exchange (1) Colloidal gold particles, passing from cytoplasm to nucleus, seem to be limited, by the annular material, to these central channels[17] , (2) ribonucleoprotein-containing particles in nucleocytoplasmic transit seem to narrow down to 100–200 Å in diameter as they squeeze through the central region of the pore complex[49], and (3) as shown here, a model based on restricted diffusion through tubular pores could account for a 2,500-fold difference in the rate of nuclear envelope permeation among solutes which differ only in molecular size If this model of the nuclear envelope as a diffusion-restricting barrier with patent pore radii of about 45 Å can be extended to include other cells, significant implications follow

Diffusable cellular metabolites and proteins with radii smaller than 45 Å move between nucleus and cytoplasm through pores at rates influenced by solute size (that is, a/r) As illustrated by plots of $t_{1/2}$ against a (Fig 6), relatively small changes in the effective radius of the solute profoundly affect rates of envelope permeation, thus providing the cell with a method of controlling the nucleocytoplasmic movement of solutes In this regard, there is evidence to suggest that the ability of cytoplasmic oestrogen receptor protein to enter the nucleus is controlled by enzymatic modification of the receptor's dimensions[10,51]

Most cellular proteins are of a size[52] that makes their movement susceptible to influence by small changes in pore radius Hence, it is possible that the envelope controls nucleocytoplasmic movement by variation of the patent pore radius Indeed, there is evidence that the patent pore radius is different in different cell types and varies within a single cell type as a function of the cell cycle and nutritional state[3] Comparison of Fig 6a–c shows that a $\pm\,10$ Å change in the oocyte pore radius would effect a two- to threefold change in the $t_{1/2}$ of ions and metabolites ($a = 5$ Å), a tenfold change in the $t_{1/2}$ of small proteins ($a = 18$ Å), and more than a 1,000-fold change in the $t_{1/2}$ of large proteins ($a > 31$ Å)

Among the most important materials transported between nucleus and cytoplasm are ribonucleoprotein particles, which are believed to be the transport forms of mRNA and rRNA These have radii greater than 45 Å and are too large to diffuse through the nuclear pores Conformational changes in the particles and/or the pore complexes would be necessary for their transport Such changes have been inferred from electron micrographs[53–56] and suggest the possibility of nuclear pore complex control of protein synthesis

Finally, as solute size approaches the dimensions of the pore, solute–pore wall interactions become increasingly important Specific site interactions, for example H-bonding and charge-charge interactions, would also then influence solute movements The demonstration that *Rana pipiens* annular material proteins have a net positive charge[45] supports the idea that factors other than size influence nuclear envelope permeability

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Sequence of the O_R operator of phage λ

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A sequence of 79 nucleotides from the λ O_R operator is obtained by primed transcription of repressor protected DNA fragments The sequence contains the primary repressor binding site plus partial duplications which can be interpreted as secondary repressor binding sites

THE λ repressor is a protein which controls the functioning of early phage genes by binding to two sites in the λ DNA known as the operators[1] Repressor bound to the operator prevents the RNA polymerase from transcribing the two early operons of λ[2] This simple model is complicated by the fact that, unlike the *lac* operator[3], the λ operator is a large and complex structure containing several repressor binding sites[4] At low concentrations, repressor binds preferentially to a primary site which is about 30 base pairs long and is located at the operator end nearest the genes of the operon At higher repressor concentrations, the remaining sites are progressively occupied, covering a total region approximately 110 base pairs in length The two operators of λ, O_L and O_R, have a similar structure but differ somewhat in their affinity for repressor O_L is reported to bind repressor about five times more tightly than O_R (ref 4)

Maniatis *et al*[5] have reported a sequence for the primary repressor binding site in the O_L operator They made use of the fact that O_L (as well as O_R) contains a site cleaved by the *Hind* II restriction endonuclease[6] Treatment of O_L or O_R with this enzyme cuts off from the operator a fragment of 30 base pairs containing the primary repressor binding site (Fig 1) Maniatis *et al* were able to sequence this fragment utilising the cleavage product containing the remainder of the operator as a primer for repair synthesis by DNA polymerase

Working with O_R, I have obtained a sequence of 80 nucleotides using RNA polymerase to transcribe operator fragments obtained by protecting the operator with repressor against nuclease digestion This paper is a preliminary report and discussion of the possible implications of this sequence which starts in the middle of O_R and extends to the end of the operator including the primary repressor binding site

O_R operator fragments were prepared in microgram amounts[7]

by digesting with pancreatic DNase the complex formed excess λ repressor and several milligrams of sonicated DN extracted from λd*bio* M30-7, a phage containing only t right-hand operator The product, collected on nitrocellulo filters, extracted and purified by gel electrophoresis, w primarily a fragment about 110 base pairs in length as dete mined by gel electrophoresis I used these fragments as template for transcription by *Escherichia coli* RNA polymeras Whether native or denatured, both strands were transcrib yielding a heterogeneous product ranging from very shc chains to chains nearly as long as the template This materi digested with RNase T_1, gave fingerprints containing a b wildering number of spots Analysis of these nucleotid revealed, however, that many of them were related and result from the heterogeneous starts and stops made by the RN polymerase Making use of nearest neighbour information and analysis with pancreatic RNase and RNase U_2, I w able to obtain the sequence of the T_1 oligonucleotides but tl heterogeneity of the product prevented further progress establishing a total sequence

Specific initiation

It has been shown by several workers[8-10] that at sufficient low nucleotide triphosphate concentrations, the RNA pol merase can no longer initiate transcription but can still elonga
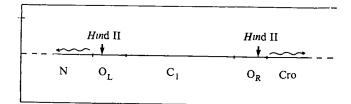
Fig 1 Schematic map of the control region of phage λ The primary repressor binding sites in O_L and O_R are located between the R *Hind* II cleavage sites (↓) and the initiation of transcription (→)
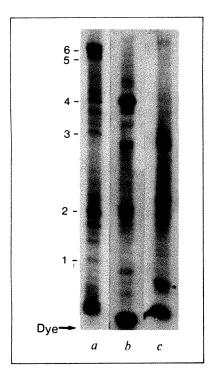
Fig. 2 Gel electrophoresis of ApApA primed transcription of O_R fragments. O_R operator fragments were prepared as previously described[7] from λd*bio* M30-7 DNA. Heat denatured fragments (about 0.2 μg) were transcribed with 5 μg RNA polymerase holoenzyme in a 50–100 μl reaction volume containing 0.05 M KCl, 0.05 M Tris pH 8.0, 0.01 M $MgCl_2$, 0.5 mg ml^{-1} bovine serum albumin, 0.1 mM dithioerythritol, 0.5 mM ApApA and 1–5 μM NTPs of which one or more were labelled with α-^{32}P at 100 μCi Mmol^{-1}. Some unprimed transcription is already detectable when the NTP concentration is as high as 5 μM. The reaction was stopped after 2 h at 37 °C by the addition of 10 μl 0.5 M EDTA and 100 μl M NH$_4$OAc and precipitated with 3 volumes of ethanol. After chilling at −70 °C the precipitate was collected, resuspended in 0.009 M Tris-Borate, pH 8.2, and 7 M urea. After heating in boiling water for 2 min, the samples were applied to a 12% acrylamide slab gel containing 0.09 M Tris-Borate, pH 8.2, 0.002 M EDTA and 7 M urea. The slab gels were run at 30 mA for 4 h or until the bromphenol blue marker had migrated 18 cm, and then autoradiographed. *a*, Transcription of whole operator fragments: the oligonucleotides contained in the numbered bands are listed in Table 1. *b* and *c*, Operator fragments were digested for 12 h with R.*Hind* II endonuclease. The products were separated by agarose acrylamide gel electrophoresis and used as templates for transcription primed by ApApA. The gel *b* shows the transcription product obtained from the larger cleavage product (approximately 80 base pairs). *c*, Shows transcription from the smaller R.*Hind* II product (approximately 30 base pairs) which contains the primary repressor binding site. The strong band near the bottom of this gel contains primarily AAAUGGUUG(C$_{OH}$), the product of incorrect priming, plus some AAAAUAGU(C$_{OH}$).

a primer. This property can be exploited to force the polymerase to initiate transcription at a given site by offering it an appropriate oligonucleotide primer[3].

The sequence information I had already obtained from unprimed transcription indicated that the operator contains a high degree of partial sequence reiteration. It was not easy therefore to find a short, unique sequence to use as primer. This problem was solved by the discovery that the polymerase has certain preferred initiation sites even on denatured DNA templates, possibly as a consequence of secondary structures assumed by the single strands. Although the sequence AAA occurs several times in the operator, the trinucleotide ApApA primes transcription starting almost exclusively at one site.

The product of ApApA primed transcription, fractionated by gel electrophoresis revealed another useful property of transcription under these conditions: the polymerase often terminates prematurely at a number of not entirely random sites, giving rise to a reproducible pattern of bands (Fig. 2a). Fingerprint analysis of these bands showed the progressive appearance of characteristic spots and facilitated the ordering of the corresponding oligonucleotides into a complete sequence. Table 1, shows the order of appearance of the oligonucleotides in the bands numbered in Fig. 2a.

Combined with nearest-neighbour analysis, these results established a probable sequence of 77 nucleotides starting with the primer ApApA. But, some details of the last 30 nucleotides remained open to alternative interpretations. These ambiguities were resolved by the observation that a minor component of the transcription product seemed to consist of sequences complementary to the principal product. Analysis of this complementary sequence resolved the ambiguities and established a sequence (Fig. 3). Surprisingly, this transcription is also primed by ApApA, initiating with AAACC... (the underlined nucleotides indicate the primer) and is clearly due to incorrect priming since the sequence should have only AACC. Transcription in the reverse direction was also obtained by priming with ApUpG, or with UpApG, yielding the sequences summarised in Fig. 3 and confirming the previous conclusions.

Location and orientation

The sequence of about 80 nucleotides shown in Fig. 3 represents the principal product of transcription primed with ApApA and, although the 3'OH end is not unique, it probably runs to the end of the operator fragment. The end is clearly somewhat ragged and can vary (at least) from position 3 to position −3 in Fig. 3 as some chains end with UpU$_{OH}$ and as priming with ApUpG starting from the end is possible in at least a fraction of the molecules.

To determine the location and orientation of the sequence in the whole operator, I prepared smaller operator pieces by
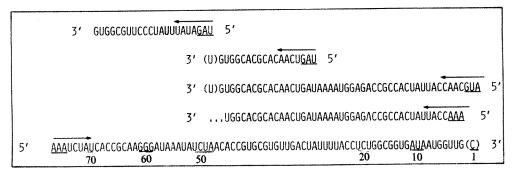
Fig. 3 Summary of the sequences obtained by primed transcription. The figure shows the sequence of the principal primed transcription products discussed in the text. The underlined nucleotides indicate the priming sequence. The nucleotides are numbered starting from the most frequently observed terminus of the operator fragment. The next nucleotide, numbered −1 is the site of transcription initiation *in vivo* (see the following article).

Table 1 ApApA primed transcription of O_R fragments

Band		Pancreatic RNase		RNase T_1
1		AAAU(C), C(U), U(A), AU(C), $\overline{AC(C)}$, C(G), C(A)		$\overline{AAAUCUAUCACCG}$(C)
2	Same as band 1 +	GC(A), AAGGGAU(A), AAAU(A)	Same as band 1 +	CAAG(G), G(G), G(A)
3	Same as band 2 +	AAC(A), GU(G)	Same as band 2 +	AUAAAUAUCUAACACCG(U), UG(C)
4	Same as band 3 +	GU(U), U(G), GAC(U), GC(G)	Same as band 3 +	CG(U), UUG(A), UG(U)
5	Same as band 4 +	U(U), U(G), GGC(G), GGU(G), U(C), AU(U)	Same as band 4 +	ACUAUUUUACCUCUG(G), G(C), UG(A), G(U), CG(G)
6	Same as band 5 +	GAU(A), AAU(G), GGU(U)	Same as band 5 +	AUAAUG(G), UUG(C)

sequence: 5'$\overline{AAA}$UCUAUCACCGCAAGGGAUAAAUAUCUAACACCGUGCGUGUGACUAUUUUACCUCUGGCGGUGAUAAU-GGUUG(C)3'

Order of appearance of oligonucleotides in gel bands. Bands were cut out from the gels using the autoradiographs as a guide. The bands were extracted by soaking overnight in 1 ml 0.5 M NH_4OAc, 0.05% SDS and 30 µg tRNA. The extract was precipitated with 3 volumes of ethanol and the precipitate was digested with RNase T_1 or pancreatic RNase. Fingerprints were obtained by electrophoresis on cellulose acetate strips in the first dimension followed by homochromatography on DEAE–cellulose thin-layer plates[19]. The spots from the fingerprints were then analysed with RNase T_1, pancreatic RNase, RNase U_2 or alkaline hydrolysis as required. The table lists only the new oligonucleotides appearing in gel bands of increasing size, but omits the 3'OH terminals. Each band also contains the nucleotides of the preceding band. The underlined nucleotides indicate the primer. The nucleotides in parentheses were deduced from experiments in which only one of the four nucleotide tri phosphates was labelled. The labelled phosphate is transferred to the 3'OH end of the oligonucleotide which precedes it.

rebinding full size operator fragments with limiting amounts of repressor and redigesting the complex with pancreatic DNase. The resulting fragments were much shorter, ranging from about 30 to about 60 base pairs. They should still include the primary binding site but should have lost some of the weaker secondary sites. Transcription of these fragments, primed with ApApA, was initiated with the AAA at position 56 in the sequence shown in Fig. 3, indicating that it proceeds in the direction of the primary repressor binding site.

An alternative way to establish the orientation of the sequence is to localise it with respect to the R.*Hin*d II cleavage site. I treated full size operator fragments with *Hin*d II endonuclease, and separated the two products by gel electrophoresis. The larger fragment, approximately 80 base pairs long, transcribed with ApApA primer, yielded a sequence which is identical to that analysed in Table 1 but terminating with UpU_{OH} after 45 nucleotides (Figs 2b and 4). Transcription of the shorter fragment, approximately 30 base pairs long, with the same primer gave a mixture of products (Figs 2c and 4). Surprisingly, the major products are the result of incorrect priming: starting with AAAUG rather than with AAUG and with AAAC rather than with AAC. Priming at the AAAA site occurs less frequently and primarily starts with the first A rather than the second, yielding $\underline{AAA}AUAGU(C_{OH})$.

I conclude from these results that the sequence obtained begins in the distal one third of the operator and proceeds to the end proximal to the operon and including the primary repressor binding site. Further confirmation of this sequence was obtained by sequencing the RNA polymerase binding site associated with O_R as reported in the accompanying article[11].

Sequence features

The position of the R.*Hin*d II cleavage site acts as a point of reference for comparison with the sequence of the primary site of O_L (Fig. 5). In O_R, the R.*Hin*d II cleavage site has the sequence 5' GTTGAC 3'. This together with the results of others shows that the R.*Hin*d II recognition sequence GTpypuAC[12] includes all four combinations of py and pu and not only the complementary pairs. There are therefore three sequences recognised by this enzyme, since GTTGAC and GTCAAC are complementary and represent the same sequence. Another reference point is the transcription initiation site which in O_R is located at the nucleotide immediately following the C at position 1 in the sequence[11]. In O_R, as in O_L, the transcription initiation is located 33 nucleotides from the R.*Hin*d II cleavage site. With these reference points the O_L primary site sequence can be compared base for base with the O_R sequence.

The results of such a comparison are at present difficult to interpret. There is a high degree of homology between O_R and O_L and many sequences occur repeatedly in O_R. Such preservation or repetition of sequence features does not necessarily signify involvement in repressor recognition, however. It might be in part the result of recognition of other proteins (RNA polymerase, *tof* gene product) which interact with the same DNA region[14,15]. Or it might simply be due to the evolutionary process which gave rise to the multiple operators and preserved irrelevant sequence features as well as important ones. In what follows I present one of several possible schemes for interpreting the O_R sequence in terms of repressor binding sites.

By comparing the O_R sequence with that of the O_L primary

```
5'  AAAUCUAUCACCGCAAGGGAUAAAUAUCUAACACCGUGCGUGUU_OH   3'          5'  AAAUGGUUG(C)   3'

                                                        3'  (C)UGAUAAAAUGGAGACCGCCACUAUUACCAAA   5'

                                                        3'  (C)UGAUAAAA   5'
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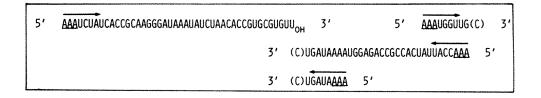
Fig. 4 Sequences obtained from the two O_R fragments produced by R.*Hin*d II cleavage. The sequence on the left represents the largest product of transcription of the larger R.*Hin*d II fragment. The sequences on the right, transcribed in both directions, are obtained from the smaller R.*Hin*d II fragment. Two of these sequences are due to incorrect priming of ApApA at sites containing only two As.
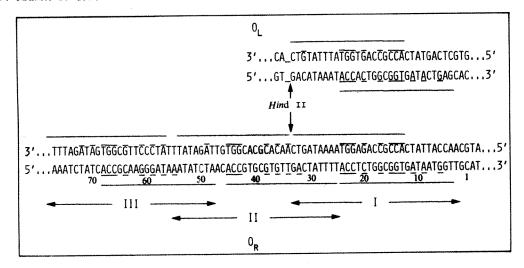
Fig. 5 Comparison of O_L and O_R sequences. Sequences in O_R comparable to the O_L sequence[5], are marked I, II and III. These partially overlapping regions are proposed as binding sites for repressor tetramers. The location of the dimers is represented by solid lines which are not intended to imply that each dimer recognises only one strand. The underlined nucleotides indicate sequence elements most nearly common to the eight dimer binding sites.

site, it is possible to single out three partially overlapping regions which present substantial sequence homology with each other and with O_L. These regions, labelled I, II and III in Fig. 5, are 30–35 base pairs long and each would correspond to a complete recognition site for a repressor oligomer, most likely a tetramer, given the size of the region involved and the structure of the repressor oligomer seen in the electron microscope (Brack and Pirrotta, unpublished). Region I, which has the highest degree of homology with O_L, is of course the primary binding site of O_R.

It has been pointed out that many recognition sequences in DNA contain a high degree of symmetry. The *lac* operator sequence contains an axis of partial twofold symmetry in which 16 out of 21 base pairs participate[3]. In the λ O_L sequence, Maniatis *et al.* have detected three such axes. In the three O_R regions which I propose, this kind of symmetry is also abundantly present. Three axes of twofold symmetry can be distinguished in region I, centred at positions 17, 18 and between 18 and 19. Similar axes occur in regions II and III at corresponding positions in their sequence. It is difficult to interpret these symmetries in terms of repressor recognition because the nucleotides involved are not always the same from one sequence to another. While this kind of symmetry would permit the formation of self-complementary loops as proposed by Gierer[17], experiments with superhelical DNA[4,18] have recently shown that such structures are not involved in repressor binding. It seems more likely instead that the symmetry of the recognition sequence simply reflects the symmetry of the protein interacting with it. Both the *lac* and the λ repressors are oligomers of identical subunits and may be reasonably expected to possess an axis of twofold symmetry. A possible binding scheme for λ repressor subunits, which exploits one set of symmetry axes and can account for the results of protection experiments, is the following.

We can image two repressor dimers binding to two regions about 21 nucleotides long and overlapping in the middle, nucleotides 4–24 and 13–33 respectively. These two regions are in fact related by a twofold axis of symmetry located between nucleotides 18 and 19, which would permit the two dimers to recognise similar sequences on opposite strands. In the O_L sequence, this interpretation would account for the second of the axes of symmetry detected by Maniatis *et al.*[5]. An additional dimer binding to O_R would occupy the region between nucleotides 25 and 45: the first half of region II. This arrangement would protect against nuclease digestion a region of approximately 45 base pairs corresponding to the $S_1 + S_2$ fragment reported by Maniatis and Ptashne[4]. Region II is the one with the lowest degree of homology with the O_L

sequence. We may expect that repressor binding to this site is weakest and stabilised only by interaction with the repressor bound at the primary site. Binding of additional repressor dimers would then fill the remaining sites, progressively increasing the size of the protected region in steps of approximately 15 base pairs. At each step, the partial overlap of the sites would permit interaction of a dimer with the preceding one.

This model yields eight sequences, two from O_L and six from O_R, which are now known and which would constitute binding sites for repressor dimers. While differing in many details, these sequences have certain features, underlined in Fig. 5, which remain remarkably constant and are nearly common to all eight proposed binding sites. According to this model, these nucleotides are therefore the most likely candidates for repressor recognition elements and for the sites of operator constitutive mutations. The only such mutation so far identified is in fact located at position 24 in O_L (ref. 5).

It will be of great interest to see whether the sequence of the remaining sites of O_R and O_L conforms to this pattern and whether other operator mutations are in fact located as predicted by the model.

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Sequence of the P_R promoter of phage λ

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The RNA polymerase binding site from the λ P_R promoter was isolated and sequenced. This DNA fragment contains the transcription initiation site and shares 25 nucleotides with O_R. The sequence preceding the initiation site suggests that the promoter recognition site is not identical with the tight binding and initiation site.

THE promoter has been loosely defined as the site on the DNA where the RNA polymerase recognises some signal which allows it to bind tightly and initiate transcription. There are several such physiologically significant sites in the DNA of phage λ, two of which are the early promoters controlled by the repressor and governing transcription of the early genes, to the right and to the left of the two operators. Genetically these promoters are located very near the operators. Some promoter mutations have been mapped between known operator mutations, suggesting that the two sites may overlap[1]. Allet and Solem[2] and Maurer *et al.*[3] have shown that certain promoter mutations alter a recognition site for the *Hind* II restriction endonuclease, which is located about 30 base pairs inside the operator. This finding implies that the operator and promoter overlap at least partially.

In the *lac* operon, the polymerase initiates transcription at the distal end of the operator and the mRNA produced contains the operator sequence[4,5], whereas in λ this is not the case. It has been shown, at least for the left-hand operon, that transcription initiates outside the immunity region[6] and in fact immediately outside the primary repressor binding site[7].

One approach to the study of the promoter has been to isolate the region of DNA with which the polymerase forms a stable complex. At temperatures above 20 °C, RNA polymerase binds extremely tightly to promoter sites, forming the so-called "open complexes" from which it is able to initiate transcription[8]. This tight binding has been exploited to isolate DNA fragments protected by RNA polymerase against nuclease digestion[9,10].

We have used the same approach to isolate a polymerase protected fragment from the vicinity of the λ O_R operator. Here we report the isolation and properties of this fragment and its sequence, obtained by primed transcription of the isolated fragment.

Isolation of polymerase protected fragments

To minimise nonspecific polymerase binding we used as starting material DNA fragments from the immediate vicinity of O_R. These fragments were obtained by sonicating the DNA of λ d*bio* M30-7, a phage containing only the right-hand operator, and selecting the operator-containing pieces by binding them' with repressor and trapping them on a nitrocellulose membrane filter. We then added RNA polymerase holoenzyme in limiting

Fig. 1 Gel electrophoresis of RNA polymerase protected fragments. Operator-containing fragments were preselected from 500 μg sonicated λd*bio* M30-7 DNA labelled with [32]P, by binding with λ repressor and filtering through a membrane filter[11]. 5 μg RNA polymerase holoenzyme were added to the preselected fragments in buffer containing 0.05 M Tris, pH 7.9, 0.1 M KCl, 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithioerythritol and 5% glycerol. After 10 min at 37 °C, pancreatic DNase was added to a concentration of 100–200 μg ml⁻¹ and incubation continued for another 10 min. If the KCl concentration was higher than 0.1 M, the DNase was not sufficiently active unless the reaction mixture was diluted. The digestion was stopped by addition of excess EDTA immediately followed by a phenol extraction and precipitation with 2 volumes of ethanol. The precipitate was resuspended and applied to a 10.4% acrylamide –0.7% agarose gel made up in 0.09 M Tris-Borate, pH 8.2, and 2.5 mM EDTA. After electrophoresis for 2 h at 25 mA, the gel was autoradiographed. *a*, RNA polymerase protected fragments; *b*, heat denatured protected fragments; *c*, operator fragments of mixed sizes prepared as previously described[11]; *d*, product of digestion of operator fragments in the presence of excess RNA polymerase. The polymerase does not protect any part of the operator in these conditions.

Fig. 2 Gel electrophoresis of transcription initiated on RNA polymerase–DNA complexes. Complexes of RNA polymerase and preselected DNA fragments were prepared and digested with pancreatic DNase as in Fig. 1. After 10 min of digestion at 37 °C, 3 unlabelled NTPs were added to the same mixture at a concentration of 0.2 mM and the fourth, α-[32]P labelled at 100 mCi μmol⁻¹, and a concentration of 5 μM. After incubation for 0.5–2 min, the reaction was stopped by adding excess EDTA and precipitating with 3 volumes ethanol. The precipitate, resuspended in 0.009 M Tris-Borate–7 M urea, was heated in boiling water for 2 min and applied to a 12% acrylamide slab gel containing 7 M urea and electrophoresed as described in ref. 14.

Table 1 Transcription initiation sequence

Pancreatic RNase	RNase T$_1$
pppAU (G), GU (A), AC (U),	pppAUG (U), UACUAAG (G),
U (A), AAGGAGGU (U), U (G)	G (A), AG (G), G (U), UUG
pppAUGUACUAAGGAGGUUG	

Bands were cut out from the slab gel and extracted as described previously[14]. The band contents were digested with pancreatic RNase and RNase T$_1$ and the products separated by electrophoresis on cellulose acetate pH 3.6 in the first dimension, followed by chromatography on PEI cellulose thin layers with 2.5 M pyridine formate pH 3.5 and 7 M urea. The sequence of the oligonucleotides were determined by analysis with pancreatic RNase, RNase T$_1$, and by nearest-neighbour information (represented in parentheses).

amounts and digested the resulting complex with pancreatic DNase. The digestion was stopped by a phenol extraction, and the DNA fragments protected against digestion, which we will refer to as polymerase-protected or P-fragments, were then precipitated with ethanol and analysed by gel electrophoresis. The P-fragments migrated as double stranded DNA about 40–45 base pairs long (Fig. 1). Heat denatured P-fragments migrate faster and are clearly distinguishable. While isolated λ operator fragments can be shown to rebind well to repressor[11,12], the P-fragments do not rebind to RNA polymerase. Nor could we demonstrate binding of repressor to the P-fragment. Furthermore, polymerase could not protect any part of the isolated operator fragments against DNase digestion. Treatment of the P-fragments with the *Hin*d II endonuclease produced no detectable change in size. While these are all negative results, taken together they suggest that if there is overlap between the repressor binding site and the polymerase binding site, it is only partial and does not extend as far as the R. *Hin*d II cleavage site.

Transcription initiation site

The RNA polymerase protected fragment contains the initiation site for transcription. We treated the polymerase–DNA complex as before with pancreatic DNase but we then added the nucleotide triphosphates to initiate transcription in the same reaction mixture. As the polymerase starts transcribing, it exposes the protected DNA to digestion by the DNase. Each polymerase complex can therefore initiate only once and from a unique site in the protected fragment. On gel electrophoresis the transcription product consists of two bands differing in length only by a few nucleotides. The largest product is 17–18 nucleotides long (Fig. 2). The fingerprints show that the two bands represent a unique sequence initiating with pppAUG (Table 1) and corresponding to the sequence of the normal transcription initiation from P$_R$ observed both *in vivo* and *in vitro*[6].

We conclude that, as was previously observed by Schaller *et al.*[13] with fd RF DNA, the site at which the RNA polymerase forms the tight complex is also the site from which it initiates transcription and that the active site of the polymerase is located at about one-third of the total distance covered by the enzyme bound to the DNA.

Primed transcription

To sequence the remaining part of the polymerase binding site we used again the RNA polymerase but this time forced transcription of the isolated protected fragments using di- or trinucleotide primers and a denatured template. Since the initiation sequence in Table 1 contains two GG sites, we used CpC as a primer, hoping to obtain transcription in the opposite direction. Gel electrophoresis of the transcription product separated several bands which, when analysed with RNase T$_1$, proved to represent a single growing chain (Fig. 3 and Table 2). The last oligonucleotides to appear corresponded to those found in the transcription initiation sequence in Table 1, showing that, contrary to our expectation, the CpC was priming in the same direction but starting near the other end of the protected fragment. The order of appearance of the RNase T$_1$ and pancreatic RNase oligonucleotides, together with nearest-neighbour information, sufficed to establish a sequence of 40 nucleotides, starting with the CpC primer and ending with the transcription initiation sequence. Since the entire protected fragment is 40–45 base pairs in length, no more than five nucleotides could still be missing. To determine these and to confirm the sequence, we attempted again to prime transcription in the opposite direction, using this time ApUpG as a primer. This primer in fact gave transcription in both directions, separable by gel electrophoresis and made it possible for us to add a maximum of three additional nucleotides to the sequence (Table 3). These were not always present and probably repesent the ragged ends left by the pancreatic DNase treatment used to prepare the protected fragments.

The promoter–operator region

The sequence we have obtained for the RNA polymerase binding site shares about 25 nucleotide pairs with the sequence of the primary repressor binding site of the λ O$_R$ operator[14]. Only the transcription initiation sequence is unique to the polymerase site (Fig. 4). The overlap between operator and polymerase site is therefore only partial and does not extend to the region recognised by the *Hin*d II endonuclease. How can we reconcile these findings with the observation that promoter mutations are located in the R. *Hin*d II site and that polymerase bound to λ DNA prevents the *Hin*d II enzyme from cleaving the operators[2,3]? We note that the latter effect was observed at a molar ratio of 30 polymerase per DNA, and could be easily explained by steric hindrance preventing the approach of the *Hin*d II enzyme or by multiple binding of polymerase in the promoter region[16]. The former observation however, implies that the polymerase recognises a region including the R. *Hin*d II sequence. It is unlikely that it could do so while bound at the initiation site since the polymerase molecule itself, as seen in the electron microscope, is not longer than the 40 base pair region it protects (Brack, personal communication). One possible explanation is that the polymerase requires additional sequence elements for recognition and 'entry' into the DNA. This would explain why the isolated protected fragments do not have

Table 2 Sequence of CpC primed transcription

Band		Pancreatic RNase		RNase T$_1$
1		C (U), U (C), U (G)		CCUCUG (G)
2	same as band 1 +	GGC (G), GGU (G)	same as band 1 +	G (C), CG (G), G (U)
3	same as band 2 +	GAU (A)	same as band 2 +	UG (A)
4	same as band 3 +	AAU (G), GGU (U), U (G), GC (A)	same as band 3 +	AUAAUG (G), G (U), UUG (C)
5	same as band 4 +	AU (G), GU (A), AC (U), U (A) AAGGAGGU (U), U (U), U (G)	same as band 4 +	CAUG (U), UACUAAG (G) G (A), AG (G), G (U), UUG

sequence 5′ CCUCUGGCGGUGAUAAUGGUUGCAUGUACUAAGGAGGUUG . . . 3′

Bands were cut out from the gel in Fig. 3 and fingerprinted as described in Table 1. Only the new oligonucleotides appearing in each band are listed. Each band contains also the oligonucleotides of the preceding band. The underlined nucleotides indicate the primer sequence.
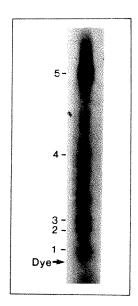
Fig. 3 Gel electrophoresis of primed transcription. RNA polymerase protected fragments were prepared as described in Fig. 1 and used as templates for transcription primed with CpC. The details of the transcription reaction and urea gel electrophoresis were as described in ref. 14. The oligonucleotide composition of the numbered bands is shown in Table 2.

appreciable affinity for RNA polymerase. We propose then that recognition, entry, tight binding and finally initiation are different elements of the polymerase–promoter interaction which need not occur at exactly the same site. The last two events clearly take place at the site whose sequence we report here. The site for the first and second events must be looked for further inside the operator sequence. In this region, however, it is difficult to discern which structural features of the sequence result from the repressor binding function and which result from the polymerase recognition. Nevertheless, by comparison with the sequence of other promoter regions we can discern two common features: First the presence of one or more AAAT sequences, and second, regions of very high GC content flanking a region of very high AT content.

Similar features have been noted in the *lac* promoter region (5) and in that of the tRNA^{Tyr} gene [16] and it has been suggested that they may be involved in the recognition and entry process. Furthermore, if the λ operator–promoter sequence is aligned with that of the lac promoter–operator region using the transcription initiation site as a reference, the GC and AT-rich regions are almost exactly in register and 22 base pairs out of 48 are identical. We suppose that a loose complex is formed by the

polymerase at the recognition or entry site. Transition of thi: loose complex to an 'open complex' might result from the open ing of some 6 base pairs, in the AT-rich region. The tight bind ing, however, occurs only when the polymerase shifts from the entry site to the binding or initiation site. This shift must occur fairly rapidly after 'opening' of the promoter and does not require the presence of the nucleotide triphosphates. We anticipate that, in the appropriate conditions, it should be possible to block the polymerase at the recognition or entry site and identify the sequence involved. Dickson *et al.*[5] have proposed a similar model for the *lac* promoter. They suggested that the role of the GC-rich regions might be to act as a "transducer"; that is to transmit the helix destabilisation produced by the catabolite gene activator protein (CAP) bound to the region immediately adjacent to the GC-rich region and so facilitate entry by the polymerase. The fact that the λ P_R promoter has the same GC–AT–GC structure even though it is not regulated by the CAP protein suggests that this structure may be a more general feature of polymerase recognition sequences. We note that in the P_R sequence the GC region contains only 73% GC rather than 83% as in the *lac* region. This difference might be enough to make polymerase entry into th λ P_R promoter possible without additional facilitation. It is possible that the transition temperature to an 'open' RNA polymerase–promoter complex is related to the GC content of the regions flanking at the AT-rich region.

Finally, we would like to point out an additional feature of the promoter–operator region, which may be significant. The sequence contains three blocks of high GC content alternating with two blocks of high AT content. Furthermore, two axes of relatively high partial symmetry are located at, or adjacent to, nucleotide 41 (Fig. 4). These two observations suggest the possibility that there may be two polymerase recognition or entry sites in this region, both composed of two GC-rich regions surrounding an AT-rich region. The two sites would share one GC-rich block, and, because of the rotational symmetry, would be oppositely oriented. We know in fact, that there exists a promoter for leftward transcription in this region. The P_{rm} or repression maintenance promoter is a weak promoter responsible for transcription of the λ C_1 or repressor gene in a lysogenic cell[17]. A mutation in this promoter has been localised in the region of nucleotides 48–54 in the O_R sequence (Hedgpeth and Smith, personal communication). Furthermore, *in vivo*, functioning of this promoter requires the presence of active repressor[17,18]. If the region between nucleotides 35 and 71 is in fact the recognition–entry site of this promoter, we can easily explain this requirement. P_R is a strong promoter: it has a high affinity for polymerase. Polymerase binding at the P_R recognition site is incompatible with binding at the P_{rm} site. Hence, in the absence of repressor, P_R transcription predominates. In the presence of repressor, P_R is pre-empted by repressor bound at the primary site of O_R, occupying the region 1–33 in the sequence.
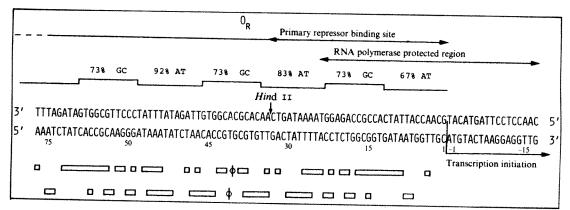
Fig. 4 Promoter–operator sequence from the O_R region. The RNA sequences are written as DNA. The regions of high GC and high AT content are marked above the sequence. The bars below the sequence indicate complementary sequences located symmetrically with respect to two possible axes of symmetry at or adjacent to nucleotide 41.

Table 3 Sequence of ApUpG primed transcription

Band	Pancreatic RNase	RNase T$_1$
1	GU (A), AC (U), U (A), AAGGAGGU (U)	AUG (U), UACUAAG (G), G (A), AG (G), G (U)
2	GC (A), AAC (C), C (A), AU (U), U (A), AU (C), AC (C), C (G), GC	AUG (C), CAACCAUUAUCACCG (C)
3	Same as in band 2 + AGAGGU (A)	Same as in band 2 + CCAG (A), AG (G), G (U), UA$_{OH}$

Sequence 3′ ^{HO} AUGGAGACCGCCACUAUUACCAACGUA 5′   5′ AUGUACUAAGGAGGU (U) 3′

The sequence of the largest T$_1$ oligonucleotide was not determined in detail but was deduced by pancreatic RNase analysis and from the sequence of the complementary strand.

This leaves P$_{rm}$ available to polymerase for transcription of the C$_1$ gene. If excess repressor is present, the additional repressor binding sites will begin to be occupied, pre-empting also P$_{rm}$ and therefore regulating by negative feedback the transcription of the repressor gene itself. A prediction generated by this model is that if the P$_R$ site is blocked by suitable amounts of repressor, or better, by cleavage with R. *Hin*d II it should be possible to detect leftward transcription *in vitro* of the O$_R$–C$_1$ region starting at the P$_{rm}$ site.

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Enhancement of the electrical excitability of neuroblastoma cells by valinomycin

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Mouse neuroblastoma cells in stationary phase of growth display partially developed electrical properties. Addition of the K$^+$ selective carrier valinomycin to these cells causes rapid enhancement of electrical excitability. We suggest that the appearance of molecules with properties similar to valinomycin is essential for the full expression of electrical excitability in differentiating neuroblastoma.

MACROCYCLIC antibiotics and related compounds which can selectively translocate ions in artificial and biological membranes, have been used to explore the molecular basis of cellular ion permeation[1-3]. The natural occurrence of ionophores in biological membranes has been reported[4,5] and supports the speculation that cyclic molecules, especially those with valinomycin-like properties, may provide the natural basis for resting membrane permeability in some tissues[3,6]. The application of compounds such as valinomycin to electrically excitable membranes, however, either did not produce detectable permeability changes[7-9], or gave rise to an impairment of electrical function[7,10,11]. As the membranes already studied possessed highly specific permeability properties, we wished to investigate an excitable system in which resting and active selectivity patterns are not fully established. Mouse C-1300 neuroblastoma, from which many clonal lines have been isolated[12,13], is particularly suitable. Cells from several lines can generate well developed action potentials after long term treatment with certain agents such as aminopterin or

dibutyryl cyclic AMP(db-cyclic AMP)[14-16], but as we show here, if these cells are maintained in the stationary phase of growth, excitability is only partially expressed. The transition from partial to full excitability, a process normally taking 1–3 weeks, includes a rise in resting potential, membrane resistivity and action potential currents. Here we show that a comparable change in electrical properties can be rapidly induced by the addition to the culture medium of low concentration of valinomycin.

Properties of stationary phase cells

Electrophysiological experiments were performed on clone N1E-115 cells in stationary phase of growth using previously described techniques for intracellular microelectrode recording[15]. Because the confluent cell population is somewhat heterogeneous, only large cells of diameter of 70–100 µm with a marked degree of process formation were studied (see Fig. 3a). As summarised in Table 1, transmembrane resting potentials and input membrane resistances were relatively low for most of these cells. When equal depolarising and hyperpolarising current pulses were applied at the resting potential, most cells showed delayed rectification, that is, smaller resistance with depolarising than with hyperpolarising pulses[17]. Figure 1a shows that with sufficiently large depolarising pulses, the elicited voltage reached a maximum, then gradually declined to a plateau 2–8 mV below the peak. As shown in Fig. 2 increments in the intensity of the depolarising pulse gave only slight

Table 1 Effects of valinomycin on electrical properties of stationary phase NIE-115 neuroblastoma cells

	Membrane potential (−mV)*	Membrane resistance (MΩ)	Time constant (ms)	Max. dV/dt spike (V s⁻¹) rise	fall	Potential at spike peak (mV)	Amplitude of fall phase (mV)	n
Control	16.4±1.4	5.5±0.5	7.8±1.0	21.1±2.9	2.3±0.4	7.6±1.7	20±1.0	37
Valinomycin treated (10⁻⁶–10⁻⁸ M)	34.3±3.8	12.5±1.8	15.3±2.1	53.6±6.8	28.5±3.2	24.7±2.0	52.5±2.0	16

Cultures were grown to confluency in Dulbecco–Vogt modified Eagle's MEM (DMEM) (GIBCO), supplemented with 10% foetal calf serum (FCS) (GIBCO) in Falcon plastic flasks. For electrophysiological studies, cells that were maintained in confluency for at least 2–4 weeks with daily changes of medium were washed twice with Puck's saline D1 and then treated with D1 containing 0.05% trypsin for 5 min. After resuspension in fresh DMEM+FCS, 5×10^5 cells were seeded per 60 mm Falcon tissue culture dish (2.4×10^4/cm²). The replated cells passed through a lag period of 4–5 d before cell division recommenced; this continued for 4–5 d until cells reached a high density confluent state (3×10^6 cells per 60 mm dish, or 1.9×10^5 cells cm⁻²). Electrical studies were made on both lag and confluent cells, their electrical properties were found to be similar. In parallel, cells from the same flasks were plated at a similar density for determination of intracellular ionic concentration by atomic absorption spectrophotometry (in preparation). Stock solutions of valinomycin (Calbiochem) at 10⁻³M were prepared in 100% ethanol, and diluted on the day of the experiment at least 1,000-fold to the concentration used in the experimental media. The ethanol concentration which never exceeded 0.2% was found to have no effect on the electrical properties of neuroblastoma cells. In the present set of experiments the membrane was exposed to a concentration of 10⁻⁸ M valinomycin after recordings were obtained from cells in normal medium. In one of two valinomycin batches used, the initial concentration of 10⁻⁸ M was found to be effective. But in the second batch, the effective concentration was 10⁻⁶M. Similar differences in valinomycin activity between batches have been seen previously and result most probably from deterioration[3,20,21].

Intracellular microelectrode recordings were made in the culture dish which was held in a special chamber on the stage of an inverted phase contrast microscope with control of temperature between 36 and 38 °C. The pH was maintained between 7.2 and 7.4 by passing a constant stream of warmed 5% CO_2 in air over the dish. The cultures were bathed in the growth medium which contained the following salt concentrations in mmol l⁻¹: NaCl-109.5; KCl-5.5; CaCl₂-1.8; MgSO₄-0.8; NaHCO₃-14.4; NaH₂PO₄-0.9. 3M-KCl micropipettes with resistance ranging from 10–20 MΩ were used. They were arranged in the bridge circuit of a Bioelectric P system to allow stimulation of the cells through the recording pipette. The transmembrane voltage and stimulating currents as well as the electronically derived time derivative of transmembrane voltage were displayed on an oscilloscope and photographed. When a stable resting membrane potential had been reached, input membrane resistance and an estimate of membrane time constant were determined using comparable hyperpolarising current pulses. Determinations of active membrane properties were made on cells adjusted to a standard membrane potential level of −95±5mV. This gives a nearly maximal rate of rise and eliminates variation as a result of differences in initial resting potential. Ionic currents flowing during an evoked action potential were quantitatively estimated from the maximum value of the time derivative of the transmembrane potential (maximum dV/dt)[15]. Responses were also measured in terms of the differences between the maximum membrane depolarisation evoked by the stimulating pulse and the adjusted steady membrane potential (potential at spike peak). Positive values of this measure indicate action potential overshooting zero potential.
*Values in all columns are given as mean±s.e.
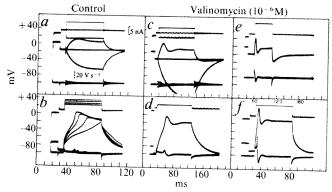
Fig. 1 The effects of valinomycin on the electrical activity of stationary-phase neuroblastoma NIE-115 cells. *a* and *b*, control records; *c* and *d*, *e* and *f*, examples of responses in the presence of valinomycin. All records are from the same dish; cells were replated 12 d before experiment. In this and subsequent figures the uppermost trace represents the injected current, inward current shown as downward deflection; the middle trace represents the transmembrane potential recorded in a bridge circuit; the lowest trace represents the time derivative of the transmembrane potential (dV/dt). Current and dV/dt calibration bars (in *a*) apply to all records. A 10 ms, 20 mV voltage calibration pulse is seen at the left of the middle traces. Records *a* and *c* represent the voltage responses to depolarising and hyperpolarising current pulses applied at the resting potential of each cell. Note that in the presence of valinomycin a smaller current intensity elicits a much larger hyperpolarising potential. In *e* an outward pulse was applied at a steady membrane potential adjusted to that of the control cell. Note the fast declining phase and hyperpolarising after potential. In *b*, *d* and *f* the steady membrane potential was adjusted to −100 mV to obtain maximal action potentials. Note that in the control (*b*), the slow time course of the falling phase is not affected by increments in the intensity of the applied current, whereas the rise rate is slightly increased; in *d* a stimulus at the threshold level was applied to show that after valinomycin treatment rise rates comparable with those in the control determine faster fall rates; *f*, a fast rising, short duration action potential followed by a slowly rising peak elicited by a larger stimulus in the presence of valinomycin.

changes in the steady potential level, whereas an almost linear current–voltage (I–V) relationship was obtained for inward currents. Few cells showed anomalous (inward-going) rectification (Fig. 3*b*)[18], or ohmic behaviour throughout the explored range.

Active membrane properties were assessed by applying depolarising current pulses at a membrane potential preset to a standard level of −95±5 mV. Under these conditions, all cells tested generated action potentials. As shown in Table 1 and Fig. 1*b*, the amplitude and rate of change of both rising and falling phases were generally low, and overshoot potentials could not always be recorded.

The electrical properties just described contrast strongly with the well developed properties obtained after long term cell selection with aminopterin or db-cyclic AMP[15,16], suggesting that a major step which is required for full expression of electrical excitability does not take place in stationary phase cells. This step may involve a change in the permeability properties of the membrane. If this change includes an increase in K⁺ permeability (P_K), it seemed possible that valinomycin, a K⁺ selective carrier[19–23], could rapidly produce an enhancement of electrical excitability.

Effect of valinomycin on passive and active membrane properties

Table 1 compares electrical properties of cells before and after treatment with valinomycin. Steady state conditions were attained about 10 min after exposure to valinomycin and remained constant for experimental periods of at least 4 h. As shown, a concentration as low as 10⁻⁸ M produced a twofold increase in average resting potential accompanied by a similar increment in input membrane resistance and a dramatic enhancement of electrical excitability.

In terms of the constant-field equation[24,25] an increase in P_K which lowers the permeability ratios P_{Na}/P_K or P_{Cl}/P_K or both can explain the observed increase in resting potential provided

mV
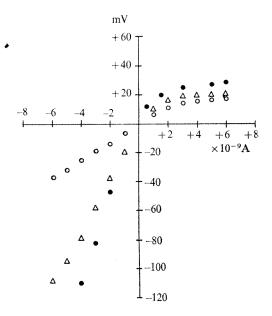
Fig. 2 Steady-state current–voltage (I–V) relationships in stationary-phase N1E-115 cells. ○, Before; ●, after addition of valinomycin (10^{-8}M) to the culture medium. Cells were replated 3 d before the experiment. △, I–V curve of a cell grown for 18 d in a culture medium containing aminopterin (10^{-7} M). The points represent the amplitude of the potential 150 ms after the start of the current pulse. Zero on the voltage axis corresponds to the resting potential of each cell which was −34 mV in all cases.

hat the internal K^+ level remains constant. Preliminary measurements of intracellular K^+ concentration in replicate plates before and after 1 h incubation with 10^{-8} M valinomycin suggest that the antibiotic does not significantly alter the internal level of this ion, in both cases values of 155 ± 5 mM were obtained.
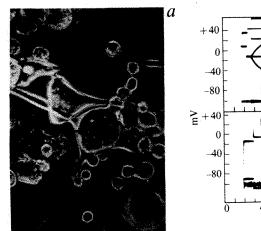
Using this value against an external K^+ concentration of 5.5 mM, an equilibrium potential for K^+ (E_K) of approximately −89 mV is obtained with the Nernst equation. This figure is substantially higher than that obtained experimentally for valinomycin-treated cells (mean value −34 mV) or that seen by us in aminopterin-selected cells (mean value −38 mV), and suggests that Na^+ and/or Cl^- ions continue to play a role in determining the resting potential.

If the hyperpolarisation of the membrane potential reflects an increase in P_K alone, then it should be paralleled by a rise in total resting membrane conductance, that is, by a fall in membrane resistance. Table 1 shows that valinomycin induces a twofold increase in the average input resistance. Thus, the steady-state I–V curve in the presence of valinomycin, resembling that obtained after aminopterin selection, exhibits a much steeper slope in the hyperpolarising direction (Fig. 2). Furthermore, although the voltage response evoked by a depolarising pulse was generally somewhat increased, as is illustrated in Fig. 3 all cells displayed delayed rectification. The unexpected increase in resistance indicates an overall diminution in the total number of passively conducting pathways. The most likely interpretation for this effect is that the fraction of membrane conductance contributed by Na^+ or Cl^- ions or both is lowered to an even greater extent than the increase in K^+ conductance.

The valinomycin induced changes in active membrane properties were of similar complexity. At membrane potentials positive to the threshold for spike initiation, when a depolarising stimulus elicited an early peak which declined to a steady-state level, valinomycin markedly increased the rate and amplitude of the decline (Fig. 1e). When spikes were initiated from the same hyperpolarised membrane potential as that of the control, the falling phase in particular exhibited a remarkable increase in both amplitude and maximum dV/dt (Table 1). This usually led to a considerable shortening in spike duration and to the appearance of an otherwise masked second, slowly rising peak (Fig. 1f). As the falling phase of the action potential is caused by an increased permeability to K^+ ions these results can be accounted for by an increased translocation of the valinomycin-K^+ complex across the excited membrane.

As shown in Table 1, valinomycin also had dramatic effects on the amplitude and rate of change of the depolarising phase. The current during this phase is carried by two systems: a tetrodotoxin (TTX)-sensitive, fast conducting Na^+ system, and a TTX-insensitive, slow conducting Ca^{2+} system[15]. The generally low maximum dV/dt values obtained with stationary cells indicate that the fast conducting Na^+ system is poorly developed at this stage. The significant increase in rise rates suggests therefore that valinomycin activates membrane elements involved in fast inward movement of Na^+ ions.

Estimation of the threshold potential for spike initiation from the inflection point between the slowly rising electrotonic potential and the upstroke of the action potential gave a value of about
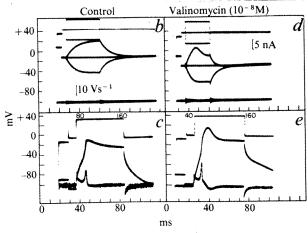
Fig. 3 Electrical activity from the same neuroblastoma cell before and after valinomycin treatment, *a*, Phase contrast photomicrograph of cells from a 'confluent' culture replated 24 d before experiment. All records were obtained from the cell designated by an arrow in *a*. Voltage responses to depolarising and hyperpolarising current pulses applied at the resting potential of the cell are presented before (*b*), and after (*d*), addition of valinomycin (10^{-8}) to the bath. Note the anomalous rectification before treatment and the delayed rectification in the presence of valinomycin. Action potentials are presented in records *c* and *e* before and after valinomycin treatment, respectively. In both cases the steady membrane potential was adjusted to about −100 mV to obtain maximal responses. Note that in the presence of valinomycin, maximum dV/dt and amplitude of both the rising and falling phases of the action potential were markedly increased. dV/dt calibration bar in *b* applies to *c*, *d* and *e*.

−40 mV both before and after treatment, so the rise in peak overshoot after valinomycin treatment represents an absolute increase in spike amplitude. Such an increase can be accounted for either by a change in electrochemical driving force for Na⁺ ions, or by an increase in the voltage-dependent changes in Na⁺ permeability during the upstroke of the action potential. Preliminary measurements of intracellular Na⁺ concentration gave a value of 35 mM before treatment which declined to 27 mM after 1 h incubation with 10^{-8} M valinomycin. Calculating E_{Na} from the Nernst equation with these figures, against an external concentration of 124.8 mM, gives values of $+34$ and $+41$ mV respectively. Because such a small increase in E_{Na} cannot account for the large differences in peak action potential amplitudes before and after valinomycin treatment, it seems that a change in the rate at which membrane permeability to Na⁺ increases during spike activity is involved.

Mode of action

Although the increase in resting potential and the enhanced repolarising electrogenesis can be easily rationalised on the basis of the known properties of valinomycin as a selective carrier of K⁺, the closing of passively conducting ionic pathways and the activation of membrane elements involved in the fast inward movement of Na⁺ ions, underline the possibility that a structural rearrangement of the membrane takes place. This may include a modification of membrane structure by valinomycin unrelated to its ionophoric function. It is also possible that the ionic selectivity characteristics of the antibiotic could be modified by such determinants of membrane organisation as surface charges, dipoles and fluidity[26]. Nevertheless, the striking similarity between the electrical properties of valinomycin-treated cells and those selected by long term incubation in aminopterin or db-cyclic AMP[14-16] and the fact that changes in K⁺ permeability seem to play a major role in many other developing cellular systems[27-30], strongly suggest that this permeability change is responsible for the rearrangement of the neuroblastoma membrane. If so, this phenomenon could shed some light on the temporal relationship between surface membrane permeability changes and other events that take place during development[31,32]. That such changes in neuroblastoma can be so rapidly triggered by valinomycin

leads us to propose that during the differentiation of these cells molecules with properties similar to those of valinomycin appear. These molecules are responsible for the induction of K⁺ permeability, an event which seems to be crucial for the full expression of electrical excitability.

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letters to nature

Non-variable 13.5 mm flux in the strong millimetre component of Centaurus A

IT has been suggested[1] that the peculiar galaxy and strong radio source Cen A has a strong and possibly variable component in the millimetre wavelength region.

Our observations do not support this claim. The evidence of variability was based on two measurements made at 3.4 mm and one at 9.5 mm at the 36-foot telescope of the National Radio Astronomy Observatory. Observations made at the position of the strong infrared source[2] were carefully calibrated against the sources of known intensity: Mars, Saturn and DR21. An important consideration in these observations was the relatively large correction for the atmospheric attenuation. At the NRAO site, Cen A has a maximum elevation of 15°. The atmospheric attenuation factor at the position of the source, $\exp(\tau)$, was about 1.5. The March 1974 measurements at 3.4 mm gave

results of 22.5 ± 2.0 Jy and 14.0 ± 1.5 Jy, and the result at 9.5 mm was 23.7 ± 2.0 Jy. If the errors cited are realistic estimates, the statistical significance of the detection of variability is very high.

We have made 18 measurements of Cen A at the position of the infrared source at a wavelength of 13.5 mm on nine days between April and December 1974. The observations were made with the 13.7-m radio telescope of the Itapetinga Radio Observatory[3]. The results of our observations of the flux density F_v are given in Table 1 with the $1-\sigma$ statistical errors, s. We also present the observations of the reference source, Virgo A, which is assumed to have a flux density of 21.4 Jy at this wavelength[4]. Although the zenith attenuation at this wavelength is greater than that cited for the NRAO work, the source is much more favourably placed in the sky for observations at Sao Paulo. The atmospheric attenuation factor was comparable to the value cited for NRAO and varied between 1.2 and 1.5.

Table 1 Flux density of Cen A and Virgo A (Jy)

Date (1974)	$F_{V_{Cen}} \pm s_{Cen}$	$F_{V_{Vir}} \pm s_{Vir}$	$r \pm s$
April 30	19 7±0 4	20 5±0 5	0 962±0 032
May 7	21 6±0 7	20 9±0 5	1 034±0 041
May 14	21 5±1 5	20 4±0 3	1 056±0 076
May 20	24 5±0 6	24 3±0 6	1 008±0 034
June 3	21 6±0 8	20 9±0 1	1 031±0 040
November 8	22 5±0 9	20 6±0 8	1 089±0 059
November 28	21 9±1 2	20 1±1 0	1 089±0 078
November 29	22 5±0 6	24 7±1 2	0 914±0 052
December 6	20 3±0 4	20 3±0 3	1 000±0 026

In Table 1 we also give the ratio r of the flux density of Cen A and Virgo A, the error s of the ratio is given by[5]

$$s^2 = s^2_{Cen}/F^2_{V_{Vir}} + [(F_{V_{Cen}}/F_{V_{Vir}})^4 (s^2_{Vir}/F^2_{V_{Cen}})]$$

A cursory inspection of the values of the ratio indicates that we have no evidence for variability at this wavelength during the times that these observations were made A simple statistical analysis equivalent to the analysis given by Fogarty *et al*[6] confirms that there is no statistical significance for the suggestion that the source might have a variable flux density at 13 5 mm

If, as it seems from our data and those of Kellermann[1], the source is variable at 3 4 mm but not variable at 13 5 mm, there must be some strongly wavelength-dependent radiation mechanism which enables the source to decrease its intensity at 3 4 mm with a very short time scale, while remaining constant at 13 5 mm

We note that the flux density of this source is approximately constant, $\approx$ 22 Jy, over the wavelength range from 3 4 mm to 13 5 mm So another interpretation of the observations is that the one measurement by Kellermann[1] of 14 0±1 5 Jy on March 28, 1974, is in error and that the source is strong but not variable

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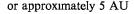
Optical variation of 3C371

WHILE studying the nucleus of 3C446 Cannon and Penston[1] put forward a hypothesis involving a small continuous source of constant luminosity which may be temporarily obscured by absorbing clouds This hypothesis is supported by our observations[2] of the nucleus of the N-galaxy 3C371, which reveal variations of its luminosity during a characteristic time of $t = 40$ min, or $2 4 \times 10^3$ s

The light curve taken on September 12, 1972, resembles those of eclipsing binary stars (Fig 1) M I Lavrov of the Kazan University (USSR) suggested (personal communication) that the luminosity changes seem to be similar to the luminosity variation of Algol It may be supposed that QSO nuclei, the nuclei of Seyfert galaxies, the nuclei of N-galaxies and other compact extragalactic objects have two or more components rotating around a common centre of gravity In this case the radius of these objects could be less than a limit given by

$$ct = (3 \times 10^{10} \text{ cm s}^{-1}) \times (2 4 \times 10^3 \text{ s})$$
$$= 7 2 \times 10^{13} \text{ cm}$$

or approximately 5 AU
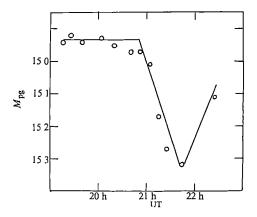
Fig 1 Light curve of 3C271

Assuming that the velocity of these objects is less than the velocity of light, their dimensions could be in the range 10^{12}–10^{13} cm, with a volume of 10^{36}–10^{39} cm^3

Setting a typical mass as 5×10^{10} $M_\odot$ their density will be some 10^5–10^8 g cm^{-3}

Such objects may resemble the hyperdense bodies required by Ambartsumian's theory[3] that galaxies and stars arise as a result of disintegration of compact objects

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Galactic gamma rays from the superposition of X-ray sources

SATELLITE observations[1-3] show an enhanced flux of high energy photons (>30 MeV) along the galactic plane The intensity has a broad and flat maximum extended $\sim$ 60° around the galactic centre The average value of the flux in the central region is $(1 1 \pm 0 3) \times 10^{-4}$ photons (cm^2 sr)$^{-1}$ in the anticentre direction it is about a factor of five less The energy spectrum in the range (35–210)MeV is quite flat and possibly indicates that the gamma rays arise from decay of π^0 mesons

Various models have been proposed to account for this flux in terms of collisions between cosmic rays and interstellar matter On these models the observations of gamma rays would give important indication on the distribution of matter and cosmic rays[4,5]

The distribution of gamma rays also resembles the distribution of X-ray sources inferred from the third Uhuru (3U) Catalog So the gamma-ray flux may result from the superposition of sources rather than having a diffuse origin This idea is not new[6-8] but the better quality of SAS-II observations compared with those of OSO-III and the larger extent 3U encouraged me to reconsider the problem

Figure 1 shows the distribution of the counts from galactic compact X-ray sources and that of gamma rays observed by SAS-II and OSO-III The distribution of the X-ray counts has been obtained by unfolding that relative to 3U sources with the same angular response function of SAS-II ($|b^{II}| < 10°$) in the regions $180° < l^{II} < 50°$, $160° < l^{II} < 180°$, and with the one of OSO-III ($|b^{II}| < 15°$) in the region $50° < l^{II} < 160°$ For variable sources I have assumed the

minimum flux (this is justified because of the small duty cycle of the flare activity) The width of the central maximum of the X-ray counts distribution is of the same order of magnitude as that reported by SAS-II for gamma rays The flux of galactic gamma rays shows secondary peaks corresponding to regions where the X-ray sources are very powerful or very numerous Nevertheless, this fit is inadequate because the ratios of the values of counts at various galactic longitude are very different for the gamma and X-ray distributions A good agreement is obtained by considering only the steady sources (Fig 2) This distribution does not take into account the sources 3U0620+23 and 3U1510-59 (possibly supernova remnants) and the anomalously strong (variable ?) sources 3U1636-53 (261±3 counts s^{-1}) and 3U1735-44 (210±6 counts s^{-1}) The source 3U1728-24 has also been excluded because it seems to be variable[8]

The dashed line shows the gamma-ray distribution The best agreement between the two distributions is obtained if one assumes that the sources emit equal amounts of energy in the X and gamma range This requires the spectrum of the sources to be very hard (a hard spectrum is consistent with an extrapolation[10] up to energy of 100 MeV of the gamma-ray emission data from Cyg X-2 in the range 1-10 MeV) or that there is an additional mechanism leading to gamma-ray emission At present the models proposed for the X-ray emission suggest thermal sources with a temperature $kT \leqslant 100$ MeV But flare-like emission at this energy could occur in the presence of strong magnetic fields with a particular configuration of the force lines In this case protons could be accelerated to energies so high as to produce, in p-p collisions, neutral mesons which in turn decay in two gamma rays

Fig. 2 ——, Distribution of counts from steady X-ray sources, – – –, distribution of gamma rays

In my opinion it is, however, premature to conclude that the gamma-ray flux observed by SAS-II and OSO-III is the result of a superposition of sources rather than to cosmic ray collisions Indeed, if X-ray sources are distributed along the spiral arms, their gamma-ray flux would have the same geometry as that resulting from cosmic ray collisions

The model could be tested by searching for time variations in the gamma-ray counts (one can reasonably expect them if gamma rays arise from flares) and by obtaining gamma-ray data with better angular resolution to bring into evidence peaks in correspondence to individual X-ray sources

I thank F Pacini for discussions

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Fig 1 *a*, Distribution of high energy (> 100 MeV) gamma rays along the galactic plane *b*, Distribution of the counts from compact X-ray sources obtained by unfolding that relative to 3U sources with the same angular response function of SAS-II and OSO-III

'Seasoning' of latent damage trails in lunar samples
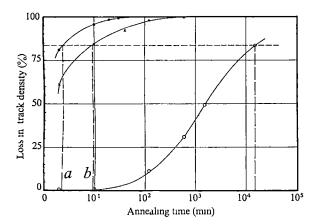
CHARGED particle track analysis has been used extensively in the study of both terrestrial and lunar samples[1-5] In the latter, the tracks are produced by both internal and external sources A careful analysis of etched track parameters not only helps in identifying various track-producing sources but also yields useful information about their charge and energy spectra Most of the lunar samples analysed[6] show that the track parameters of iron group nuclei from solar and galactic sources are very different from and more stable than those produced by (for example) 9 6 MeV per nucleon ^{56}Fe ions in simulation experiments in the laboratory Similar effects are expected for other charged particle tracks It seems as though the latent damage trails in lunar samples have been 'seasoned' or their structures have been so modified by lunar conditions (particularly, lunar temperature and high doses of light charged particles) that significant changes are brought about in their properties

Thus, to interpret correctly the results of charged particle track analysis of lunar samples, one needs to study the

characteristic differences between naturally 'seasoned' tracks in lunar samples and 'unseasoned' tracks produced in the laboratory Here we describe a study of the relative roles of temperature and time in the process of 'seasoning' Attempts have been made to 'season' or to 'stabilise' the latent damage trails by annealing the samples under various combinations of temperatures and time intervals We also give a comparison of the annealing properties of 'seasoned' and 'unseasoned' damage trails

A big piece of tektite glass was exposed to thermal neutrons to produce induced fission of uranium atoms present in it as an impurity The piece was then cut into a number of slices On polishing and etching the slices, they were found to have a track density of about 10^6 tracks cm^{-2} The uranium distribution in the glass was extremely uniform The slices were then broken further into small parts, and some of them systematically annealed at temperatures of 300 °C, 500 °C and 550 °C for time intervals varying from 2 min to $1\,6 \times 10^4$ min, 6×10^2 min and 3×10^1 min respectively The purpose of these investigations was to study the track annealing properties of the glass and the characteristic differences between 'long term' and 'short term' annealings The slices annealed at 300 °C for $1\,6 \times 10^4$ min simulate the production of the 'seasoned' or 'stabilised' tracks, those annealed at 500 °C and 550 °C for 600 and 30 min respectively produce 'unseasoned' tracks The loss in track density (expressed as a percentage of the unannealed track density) is given as a function of the annealing time in Fig 1 To produce the same degree of annealing in samples exposed to different temperatures, they were to be annealed for such times that equal losses in track densities were obtained for all the samples Figure 1 shows that to have a loss in track density of (say) 84% for the samples annealed at 550 °C, 500 °C and 300 °C, we need annealing times of 2 3, 9 3 and $1\,6 \times 10^4$ min respectively In practice, the sample annealed at 300 °C for $1\,6 \times 10^4$ min (sample A) was kept for further experiments, while two new samples (B and C) were annealed afresh at this stage Thus all the three samples contained fission tracks annealed to the same degree but in different modes sample A contained 'seasoned' tracks while B and C contained 'unseasoned' tracks All the three samples were annealed later at 300 °C for time intervals ranging from 2 min to $1\,6 \times 10^4$ min, and the loss in track density as a fraction of the 16% tracks surviving after the first annealing step (Fig 1) was plotted as a function of annealing time (Fig 2) Although there is no appreciable difference in the residual track densities for shorter annealing times ($\lesssim 10^2$ min), the longer annealing times do show a marked difference in the stabilities of 'seasoned' and 'unseasoned' tracks—the 'seasoned' ones being the more stable

Fig 1 The annealing pattern of (neutron induced) 'fresh' fission tracks in a tektite glass as a function of the temperatures and time intervals used ●, 550 °C, ○, 300 °C, ×, 500 °C
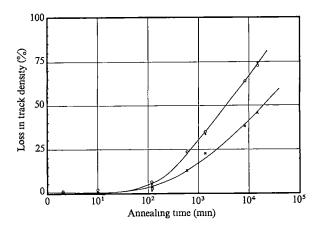
Fig 2 The annealing behaviour of 'seasoned' and 'unseasoned' fission tracks in a tektite glass as a function of time at a fixed temperature of 300 °C 'Seasoned' tracks are more stable ●, 550 °C, ○, 500 °C, ×, 300 °C

So annealing temperatures and their duration are important for the redistribution of the damage pattern during the 'seasoning' process For example, a latent damage trail annealed at a low temperature for a long interval of time (conditions similar to lunar environment) readjusts itself to a more stable state than if it is subjected to a high temperature for a short interval of time Thus, the degree of annealing is not the only important parameter in controlling the redistribution of the damage pattern, but the 'mode of annealing' plays an equally important role This implies that the etched track parameters of 'seasoned latent damage trails' (as mostly found in lunar samples) and the 'unseasoned ones' (as obtained in the laboratory) will differ significantly Their direct comparison is not justified and more work is necessary on the two types of tracks before any firm conclusions may be drawn

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Molecular stratigraphy

ORGANIC molecules contained in sediments were called 'chemical fossils' by Eglinton and Calvin[1], and Calvin[2] has used the term "molecular palaeontology" to emphasise the biological heritage of those components They recognised the important concept that molecular organic components in sediments may be viewed in much the same way as morphological remains of organisms Others have rigorously examined this concept and solidified its basis[3-6] We report organic geochemical data on some Pleistocene sediments, correlated by physical stratigraphic methods, which indicate

Table 1 Samples and extraction data

Sample	Lateral distance* (km)	Dry weight (g)	Extractable proportion (%)†	Proportion of heptane eluate (%)
Mono Basin				
1-L-76		17 5	4 70	0 79
	2 4			
1-L-76(10B)		15 4	3 84	1 97
Searles Basin				
Core B-Bed T2b		23 9	1 81	‡
	2 6			
Core C-Bed C-6		28 4	1 20	1 80
Core D-Bed D-4		31 8	0 87	1 38
	3 4			
Core E-Bed E-4		30 2	1 28	1 37

* Between stratigraphically correlated samples
† On dry sample weight basis
‡ Abundant sulphur (eluted with heptane fraction) irreversibly precipitated on concentration

that the organic composition of sediments may be utilised as a correlation parameter comparable to morphological fossils in biostratigraphy The samples we have chosen for study are lacustrine sediments from two Pleistocene basins of eastern California

Mono and Searles Lakes are two of several strongly alkaline desert lakes of eastern California Mono Lake is in a closed drainage basin, with neither an outlet to the sea nor a sink The lake is now desiccating[7-10], although it is not yet sufficiently concentrated to deposit salts Mono Basin contains a sequence of Pleistocene lacustrine silts and diatomites approximately 1 5 km thick[8,11] Arching of the
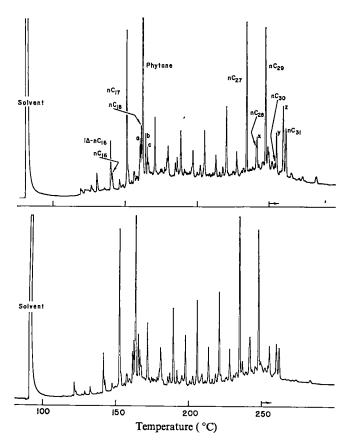
Fig 1 Gas chromatograms (nC$_7$ fr) of time equivalent sediment samples, Mono Basin, California The two samples were collected from measured stratigraphic sections located 2 4 km apart (Analysis was on a 15 m×0 5 mm support coated open tubular column, coated with SE-30, helium carrier gas flow 4 6 ml min^{-1}, temperature programmed 80–250 °C at 2 °C min^{-1}, injector temperature 275 °C, detector temperature 295 °C.)

bottom of the lake, forming Paoha and surrounding islands occurred late in the development of the basin[8][12] Detailed stratigraphic columns of Late Pleistocene sediments, given the informal designation Paoha Island Sequence, were measured on Paoha Island The sediments are generally finely-laminated algal silts, diatomaceous silts or silty diatomites, with some sands through the section, and rare basaltic and rhyolitic tuffs[13] The samples from Mono Basin were collected from measured stratigraphic sections 2 4 km apart Correlation of the two measured sections is based on lithologic sequences

Searles Lake is the dry vestige of one of a chain of four Pleistocene Lakes[14,15] Searles Basin contains a remarkable sequence of Pleistocene salts and muds, approximately 1 km thick[16], salts and brines from the basin are being mined on a large scale Kerr–McGee Chemical Corporation donated four core segments (cores B, C, D, E) of the Parting Mud[1] cores B and C were drilled 2 6 km apart, and cores D and E 3 4 km apart The sediments consist primarily of finely laminated clays, occasionally with intercalated thin lamina of various carbonate minerals The presence of aragonite dolomite, pirssonite and gaylussite has been documented by X-ray diffraction Large (>2 cm), apparently diagenetic crystals of pirssonite and gaylussite, oriented by their 'c axes at high angles to bedding, are common in parts of these cores We found no sand in the section studied Stratigraphic correlation between drill cores is based on thin (2–3 mm rhyolite tuffs, now largely zeolitised to phillipsite, which occur near the top of the Parting Mud

We used a mixture of benzene and methanol (3 1 v/v to extract the organic components from dried, powdered sediment samples The concentrated extract was fractionated by liquid–solid chromatography on silica gel, successively eluting with heptane, 5, 10, 15 and 20% (v/v) ethyl acetate in heptane, ethyl acetate and methanol The heptane eluate (nC$_7$ fr) was analysed by gas–liquid chromatography (GLC) After initial GLC analysis, the heptane eluate was segregated further into three component fractions using silver nitrate impregnated thin-layer chromatographic plate (silica gel G, 0 25 mm, 15% AgNO$_3$ in methanol, mobil phase heptane–benzene=99 1) After recovery each of these three fractions was again analysed by GLC, with authentic compounds coinjected The final analytical procedure was combined gas chromatography–mass spectrometry (GC–MS on a DuPont 21-491 mass spectrometer

For this work we have sampled time-equivalent strata at widely separated locations Mono Basin samples 1-L-76 and 1-L-76(10B) represent exact lateral equivalents, in the Searles Basin samples, Bed T2b (Core B) is the exact lateral equivalent of Bed C-6 (Core C), similarly Bed D-4 (Core D corresponds to Bed E-4 (Core E) The total proportion of extractable material in a sediment and the concentration of hydrocarbons in that extract has been used in the past to judge similar organic compositions[17] Our extraction data (Table 1) for precisely correlated beds within a sedimentary sequence indicate that this criterion may be uncertain

Stratigraphic variability in organic composition for sediments from Mono Basin has already been documented[13][18] Figure 1 shows that sediment samples of the same bed collected from localities 2 4 km apart, possess an identical distribution of individual molecular components Phytane (2,6,10,14 tetramethylhexadecane), nC$_{17}$, nC$_{27}$, and nC$_{29}$ are the dominant compounds in the hydrocarbon fraction of these samples The homologous series of n-alkanes has an attendant homologous series of mono-olefins, one of which is labelled (1Δ-nC$_{16}$) on the figure Peak a represents a composite of two components, one an unidentified branched alkane, the other being 1Δ-nC$_{18}$ Identification of peaks b c, x, y, z is still unsubstantiated

Figure 2 also illustrates lateral continuity in organic composition, although two stratigraphic horizons in Searles Basin have different compositions, each unit laterally ex
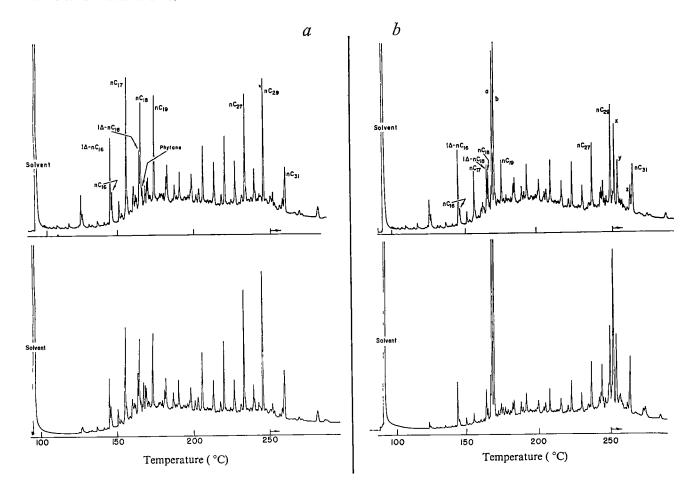
Fig 2   Gas chromatograms (nC_7 fr) of time equivalent sediment samples, Searles Basin, California Analytical conditions as for Fig 1
a, Bed T2b (core B) is the exact time equivalent of Bed C-6 (core C), the core locations are 2 6 km apart These samples represent
the interval 1–4 mm below Tuff II *b*, Bed D-4 (core D) is the time equivalent of Bed E-4 (core E), the core locations are 3 4 km apart These
samples represent the interval 778–790 mm above Tuff I

hibits a consistent composition The series of mono-olefins observed in the previously discussed samples is more prominent in these sediments from Searles Basin For the stratum correlated between cores D and E, the two most abundant constituents (Fig 2*b*, peaks a and b) are mono-olefinic *iso*prenoids (peak a   3,7,11,15 tetramethylhexadec-1-ene, peak b   3,7,11,15 tetramethylhexadec-2-ene) The mass spectra for these two components were identical, assignment of the double bond position is based on GLC retention times[19] Identification of peaks x, y, and z is still unsubstantiated, peak x seems to represent a mixture of two $C_{28}H_{46}$ sterenes, and peak z a mixture of two $C_{30}H_{50}$ pentacyclic triterpenes

Therefore we agree with Eglinton and Calvin[1,2] on the fossil nature of some organic molecules in sediments We suggest the new term 'molecular-stratigraphy', to convey the qualification that not only are organic molecules inherited from biota, but that these molecules must be constrained by biostratigraphic principles such as facies Lateral variation in the molecular organic composition of a stratigraphic unit may result either from ecologically controlled distributions of biota, or from selective preservation (over- or under-representation of components depending on the character of the material being fossilised with respect to depositional regime) The lacustrine sediments we have examined in this study grade laterally into alluvial sedimentary sequences The organic composition of the alluvial facies no doubt will be different from that of the lacustrine facies In this work we selected time-equivalent samples

representative of the same depositional facies In sedimentary sequences where fossils are scarce, molecular stratigraphy may represent the only correlation parameter available

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Electron spectroscopic observations on extreme pressure lubrication

COMPOUNDS of sulphur, phosphorus, zinc and chlorine are added to lubricating oils to control wear under arduous conditions by forming films on rubbing surfaces The films are extremely thin and frequently amorphous Only rarely has it been possible to identify compounds present although electron probe microanalysis can show the distribution of elements We here present new information, obtained from electron spectroscopic measurements, mainly concerning the binding energies of photoelectrons (ejected with $AlK\alpha$ X rays) the magnitudes of which are characteristic of particular atoms and electrons, and which may therefore be used for analytical purposes We also made some observations of the kinetic energies of Auger electrons, which are also well defined and of analytical value These energies depend on the charge on the atom, so that for states of combination of an element where the charges on the atoms are sufficiently different, valuable 'chemical shifts' are observed, sulphur is a favourable case with a shift of about 6 eV from sulphide to sulphate Only electrons that escape without collision are of value, so the analysed depth was limited to a few tens of Å, the region of interest in lubrication

Specimens of EN31 steel, after diamond polishing and scrupulous solvent cleansing, were treated in solutions of additives in non-polar white oil They were either statically immersed for 16 h at a temperature of 145 °C, or rubbed for 10 min against a disk of the same material in a wear machine Before examination in the electron spectrometer the specimens were washed in non-polar solvents to remove oil

The surface of a specimen immersed in a solution of elemental sulphur in white oil showed S2p peaks, denoting binding energies of 168 8 and 164 eV The first peak value was greatly diminished by washing the specimen in water, it matched that of a thin layer of sulphate on iron The second value was reasonably close to that obtained for elemental sulphur The peak value was not, however, much reduced by washing in carbon disulphide, so reservations must be made in attributing it to sulphur The rubbed specimen showed much less sulphate but gave a large signal corresponding to sulphide, and part of the surface oxygen was seen from the oxygen 1s spectrum to correspond to oxide We conclude that whereas the specimen which had been immersed had a rather uniform coating of iron sulphate, the surface of the rubbed specimen was at least partially oxidised and carried a relatively thick but discontinuous layer of iron sulphide

A specimen heated in a solution of dibenzyl disulphide in white oil gave peaks in the same positions as the sulphur solution Again, that which indicated a binding energy close to 169 eV was very sensitive to washing in water, and we therefore attributed it to sulphate The interpretation of that at approximately 164 eV is less straightforward in that the additive itself gave a very similar binding energy We found, however, that dibenzyl disulphide could be removed by the solvent washing It was also sensitive to washing in water unlike the substance on the specimens The peak at 164 eV cannot, therefore, be attributed to dibenzyl disulphide Dibenzyl monosulphide, a proposed intermediate[1-2] which has a closely similar binding energy, can be ruled out because it volatilises in the vacuum of the spectrometer Benzyl mercaptide can also be ruled out because it would give a peak in the ferrous sulphide position As in the previous experiment, it is tempting to assign this peak to sulphur, but there must be reservations

The specimen rubbed in the white oil solution of di-benzyl disulphide also behaved very like that in the sulphur experiment, showing mainly sulphide sulphur, with some sulphate, and oxygen present in part as oxide

Most of our work has, however, been done with a commercial zinc dialkyldithiophosphate additive in which the alkyl groups .are predominantly methylpentyl This additive contains 13% mineral oil Pure zinc di-n butyldithiophosphate was also examined

Specimens immersed in solutions of the commercial additive in white oil had zinc, phosphorus and sulphur on the surface, the sulphur was in two forms, the more abundant of which corresponded to sulphate The zinc phosphorus and the minor sulphur component displayed electron binding energies indistinguishable from those of the original additive The binding energy of that sulphur was also the same as that of sulphide sulphur Electron spectroscopy alone, therefore, could not distinguish between the two possibilities that the minor sulphur was present as a sulphide film or was present together with the zinc and phosphorus in a layer of adsorbed additive The second possibility was eliminated by re-examining the specimens after they had been washed in boiling acetone This treatment, which supposedly[3] removed adsorbed additive, did not change the spectrum Although the possibility that a sulphide film is present is not completely out of the question it seems more likely that the minor sulphur is bound up, together with the zinc and phosphorus, in a decomposition product of the original additive The evidence for this is the relatively small variation of the atomic ratios of zinc phosphorus sulphur found in our experiments over a range of conditions This ratio is, for example, 1 1 0 6 at 140 °C, whereas that for the original additive is 1 2 4 Zinc phosphate, zinc sulphide and zinc sulphate were not present and the presence of zinc oxide also seems unlikely from a consideration of Auger line widths These observations, together with others not reported here, suggest that the reaction mechanism varies with temperature and that a complex, possibly polymeric, phosphate forms on the metal surface on which there is also iron oxide and possibly iron sulphide or sulphate The pure di-n-butyl compound gave much less sulphate on the surface, which suggests that in the commercial additive either impurities which contained sulphur or sulphur contained in the mineral oil carrier contributed largely to sulphate formation

As with the sulphur additives, the specimens rubbed in the solution of the commercial zinc dialkyldithiophosphate showed a much smaller sulphate peak, but otherwise the spectra were rather similar to those of the immersion specimens The atomic ratios of zinc, phosphorus and sulphur showed less variation and more of the surface was iron oxide

Other investigators have suggested that it is possible, under various conditions and at various temperatures, that adsorbed additive iron sulphide and iron benzyl mercaptide are responsible for the anti-wear performance of the dibenzyl disulphide (refs 1 and 2, and R C Coy and T F J Quinn, unpublished) The literature on zinc dialkyldithiophosphates proposes many reaction schemes, often unsupported by analytical evidence, in which zinc oxide, zinc sulphate, zinc phosphate and polymers with definite zinc–phosphorus–sulphur ratios of 1 2 4 to 1 2 2 are postulated (see refs 3–5)

The methods of electron spectroscopy clearly provide new opportunities for progress in the study of lubrication phenomena In particular, they have given information on very thin films on rubbed surfaces—films which have been too thin to examine by other means Additionally, by showing large differences in the compositions of films on immersed and rubbed specimens, they indicate the dangers inherent in basing reaction mechanisms on immersion tests alone

A fuller account of this work will be published elsewhere

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table cold cathode arc

HE cold cathode arc, so called because of the very high current ınsities and low temperatures observed at the cathode, has ıen subjected to ıntensıve theoretical and experımental study[1-4] Iany theories of the 'cathode spot' have been proposed, but ıstınguıshıng between them experımentally has proved to ɛ a formıdable task, because of the rapıd and unstable motıon f the spot, and the large pressure and temperature gradients ıstıng wıthın a very short dıstance of the cathode surface

It is possible that a caesıum discharge run at temperatures ɔ to 1,200 K wıth a tungsten cathode, and with the entıre .scharge (not just the electrodes) heated externally, may ɛhave as a stable, cold cathode arc The low ıonısatıon otentıal of caesıum (3 9 V) could allow enough plasma by ıermal ıonısatıon for measurable currents to be obtaıned, nd the work functıon of tungsten (4 5 eV) may be hıgh enough ɔr cold cathode behaviour to occur

The discharge tube consısted of a commercıal, hıgh pressure, ɔdıum arc lamp, wıth the arc tube fılled wıth 0 1 g of caesıum ıstead of sodıum The arc tube was made of 8 mm diameter lumına, wıth a space of 9 cm between tungsten electrodes f 1 cm² surface area There was a sılıca-glass outer tube, nd the space between the tubes was fılled wıth argon at a ressure of 200 mm Hg The device was enclosed ın a cylındrıcal ırnace capable of producıng temperatures of up to 1,400 K, ıth an adjustable temperature control, and wıth ıts axıs ıclıned at about 20° to the horızontal Tungsten wıres to the lectrodes of the device were brought out of the furnace ın ın, sealed, sılıca-glass tubes Temperatures of up to 1,100 K ıere achıeved wıthout damage

Before measurements were taken, the posıtıon of the arc ıbe relatıve to the temperature gradient along the furnace ıigher temperature at the hıgh end) was adjusted untıl lıquıd aesıum was observed to condense only at the colder end of he arc tube The temperature of the caesıum gas was taken ɔ be that measured by a thermocouple near the hotter end ıf the tube The temperature of the caesıum lıquıd (whıch ontrolled the gas pressure) was estımated to be 5% lower Kelvın) than the gas temperature, whıch corresponded wıth he temperature measured by a thermocouple near the colder nd of the tube

A typıcal plot of current agaınst voltage (Fıg 1) shows that or both current dırectıons, there ıs a proportıonal ıncrease ıf current above about 8 V, indıcatıng a constant electrode ɔltage, and a constant arc column resistance The slope of he straıght lıne (Fıg 1) gıves the resistance of the column, ınd ıts ıntercept on the voltage axıs gıves a measure of the lectrode voltage The electrode voltages obtaıned when the ɔlder electrode was used as cathode were discarded, as they ended to vary (presumably dependıng on the amount of ontact between the tungsten electrode and the lıquıd caesıum) The electrode voltages obtaıned when the hotter electrode was ,sed as cathode, however, were relıable and repeatable and he shapes of the curves on ınıtıal applıcatıon of voltage
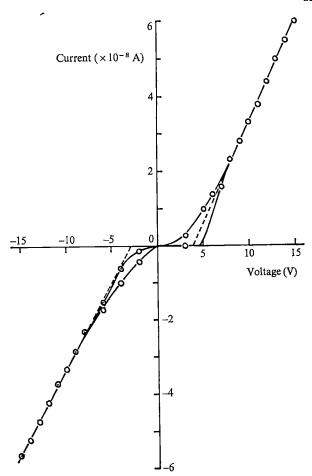
Fig. 1 Typıcal plot of current agaınst voltage Gas temperature, 760 K Posıtıve readıngs for hotter electrode as cathode

(Fıg 1, the lower curve) and on subsequent applıcatıons (Fıg 1, the upper curve), seemed to rule out thermıonıc emıssıon

The electrical conductıvıty, σ, of the caesıum column was derıved from the resistance measurements Log σ was then plotted agaınst the recıprocal of the gas temperature, T, the result was a straıght lıne (Fıg 2) The electrode voltage ob-taıned when the hotter electrode was used as cathode was also plotted agaınst $1/T$ (see Fıg 3) It was found that the experı-mental poınts could be fitted to an exponentıal curve (Fıg 3) with an asymptote at 2 65 V

Above a gas temperature of about 970 K (~1 atm gas pressure), no arc could be obtaıned, and the current dropped below the measurıng lımıt of the apparatus ($<10^{-10}$ A) Be-tween about 770 and 970 K, the arc could be made to run, and when ıt did run ıt gave repeatable results, but ıt showed a tendency to extınguish or not to start at all Below about 770 K the arc would always run

The standard formula for the electrical conductıvıty of a weakly ıonısed gas ıs $ne^2/n_a mcq$, where n ıs the plasma number densıty, n_a the gas number densıty, and e, m, c and q, respec-tıvely, the electron charge, mass, thermal speed, and collısıon cross sectıon for momentum transfer wıth neutral partıcles Assumıng thermodynamıc equılıbrıum condıtıons, Saha's equatıon may be applıed[2] to yield $n^2 \alpha n_a \exp(-e\bar{V_i}/KT)$, where $\bar{V_i}$ ıs the effectıve ıonısatıon potentıal, and the dependence of the constant of proportıonalıty on temperature can usually be neglected ın comparıson wıth the rapıd varıatıon of the ex-ponentıal term wıth temperature Thus, the electrical conduc-tıvıty should obey a relationshıp of the form

$$\sigma = \sigma_0 \exp[-e(\bar{V_i} - V_{evap})/2KT]$$

where $n_a \alpha \exp(-eV_{evap}/KT)$ ıs used, wıth V_{evap} ıncreased by
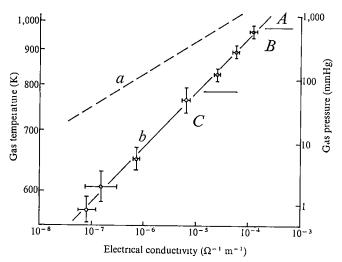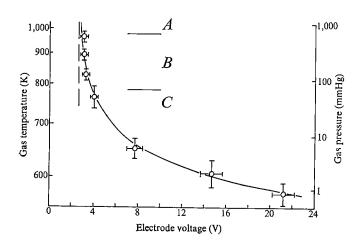
Fig 2 Relationship between gas temperature and pressure and electrical conductivity *a*, Theoretical line assuming no wall/electrode excitation mechanism *b*, experimental line *A*, region of no arc, *B*, region of marginally stable arc, *C*, region of stable arc

5% from its standard value, to allow for the fact that the caesium liquid temperature is 5% below the gas temperature

The dashed line in Fig 2(*a*) is obtained by substituting $\bar{V}_i = V_i = 3\,9\,V$ and $q = 1\,5 \times 10^{-14}\,cm^2$ into the formula for σ Clearly, straightforward volume ionisation is not the dominant mechanism The experimental line (Fig 2*b*) corresponds to $\bar{V}_i = 2\,64\,V$ and $q = 4\,10^{-12}\,cm^2$, with estimated errors of $\pm 0\,2\,V$ on $\bar{V}_i$ and up to a factor of 3 on q This result is readily interpreted in terms of a dominant mechanism involving excitation at the walls or the electrodes of the discharge The lowest excitation potential of caesium, V_{exc}, is $1\,4\,V$, so that the voltage required to ionise excited atoms is $(V_i - V_{exc}) = 2\,5\,V$, agreeing with the measured $\bar{V}_i$ within the experimental accuracy In fact, at these temperatures, a very long time is required[2] to achieve volume thermal ionisation, so any appreciable wall or electrode mechanism could be expected to dominate Furthermore, the very large experimental cross section (even allowing for the large possible error and the fact that the gas is caesium) provides a strong indication that the neutral particles are likely to be excited atoms or molecules

Fig 3 Relationship between gas temperature and pressure and electrode voltage Experimental points are for the hotter electrode as cathode The smooth curve is an exponential curve fitted to the experimental points, with asymptote at 2 65 V Regions *A*–*C* as for Fig 2

The asymptotic value of electrode voltage, 2 65 V (Fig 3) is also consistent with an explanation in terms excited atoms Allowing for a small drop in anode volt $(\sim KT/e)$, the asymptotic cathode voltage is again very cl to $(V_i - V_{exc})$ Robson and von Engel[5] have proposed a mech ism which seems to account for these observations Exc atoms impinging on the cathode release secondary electr with a high yield The net energy required for an electron escape is $e(V_i - V_{exc})$, and this determines the cathode volt required

The exponential curve fitted to the electrode voltage readi in Fig 3 may be expressed in terms of gas number densit

$$V = 2\,65\,[1 + (n_0/n_a)^{2/3}]$$

where $n_0 = 4 \times 10^{17}\,cm^{-3}$ In terms of the interparticle sep tion distance for neutrals $\lambda_a = n_a^{-1/3}$, this becomes

$$V = 2\,65\,[1 + (\lambda_a/\lambda_0)^2]$$

where $\lambda_0 = 1\,4 \times 10^{-6}\,cm$ Now λ_0 is about twice the s length for the Schottky inverse square law image force act on electrons associated with the surface potential barr Thus, the experimental result implies that when the neu interparticle separation distance increases to a value compara to the range of the surface potential barrier, more cath voltage is required to pull electrons clear of the surface T again is entirely consistent with the Robson–von En explanation

What is the reason for the arc failing to run above 970 One possibility is that volume ionisation and thus (by principle of detailed balancing) volume recombination beco more important than surface effects above this temperatu and the discharge reverts to full volume equilibrium But dashed line in Fig 1 suggests that even if that were to oc there should still be an easily measurable current It would a be necessary for a volume mechanism for quenching exci states to become important[2] That would suppress the emissi process suggested by Robson and von Engel and the curr would then drop to the thermionic emission level for tungst below the minimum that could be detected

Finally, a 'unipolar' arc effect was also observed Wh the arc was run for long periods (30 min or so) in one directi it could be observed to charge up, just like a battery It wo then deliver current to a resistive load for a comparable peri The 'e m f' associated with the effect was about 1 8 V T may have been caused by a monolayer of caesium ions hering to the tungsten electrode, as has been observed Langmuir and coworkers[6] at much lower pressure, but if then it must have been formed at the anode during the chargi process, and not at the cathode If it had been formed at t cathode, it would have caused current to continue in the sai direction after removal of the supply voltage, contrary what was, in fact, observed

I thank F J L Crammond and his colleagues at Tho Lighting, Leicester, for invaluable assistance with a diffici apparatus, and J E Allen for helpful discussions which l me to try this experiment

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Electromagnetic effects at metallic fracture

JUST at the instant of tensile fracture of low-carbon iron specimens a transient magnetic field of the 'order of 1,000 gauss is produced[1]. This phenomenon is observed even in the absence of any external magnetic field. Only the surface region of the fractured pieces are magnetised, with the fractured ends exhibiting distinct and opposite polarities. The magnetisation of each piece varies along the length, with maximum at the fractured end. Moreover, the induced magnetisation is retained for about 15 d in larger specimens and then decays almost exponentially. Iron specimens fractured under impact forces did not show any magnetisation. I now report observations of the complementary phenomenon of generation of an electric field at the instant of tensile fracture of metallic specimens, ferromagnetic as well as non-ferromagnetic.

Metals investigated, were 0.15% carbon steel, aluminium (4.5% Si, 0.5% Fe, 95% Al), brass (63.5% Cu, 0.1% Pb, rest Zn), zinc (99.67% pure) and lead (99.43% pure). The ultimate tensile strengths of these materials were of the order of 39.0, 29.4, 33.2, 6.2 and 2.0 kg mm^{-2}, respectively. The zinc and lead rods were processed by metal mould casting and the others by hot rolling.
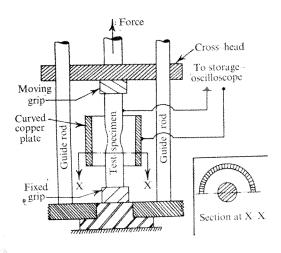
Fig. 1 Schematic diagram of the force application system of the tensile testing machine.

External electrical and magnetic disturbances (including the Earth's field) were shielded out during the experiments. Test specimens were stressed in vertical and horizontal directions, and also in different orientations with respect to the magnetic meridian. A coaxial, semicylindrically curved copper plate (30 mm internal diameter and 50 mm long) was used as an electric field sensor. A Hewlett Packard variable persistent storage oscilloscope was used to record the phenomenon. Measurements of potential difference were also made with the help of a d.c. microvoltmeter. The potential pickup wire soldered on to the outer surface of the curved copper plate was connected to the storage oscilloscope and the ground wire, first direct on to the surface of the test specimen with the machine body grounded separately and then with the ground wire of the oscilloscope connected to the machine body without disturbing the test specimen, the latter being at ground potential by contact with the machine parts. The curved copper plate did not suffer any mechanical jerk resulting from stressing and fracture (Fig. 1).

Test specimens had dimensions 9 mm diameter × 160 mm long and were fractured under tension in a horizontal Housfield Tensometer. The test specimens were machine turned to a
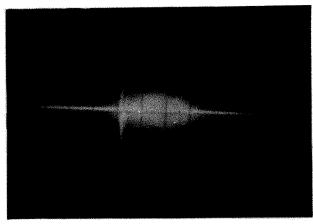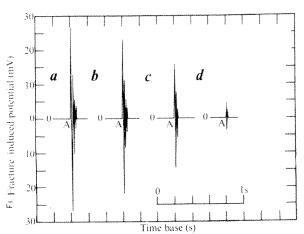
Fig. 2 Photograph of the voltage against time plot obtained on the storage oscilloscope for an aluminium specimen.

diameter of 7 mm in the central zone (Fig. 1) to ensure fracture in the centre. The transient magnetic fields in ferromagnetic specimens at the instant of tensile fracture were measured simultaneously as in my earlier work[1]; the results agree with those of that study.

Figure 2 is a photograph of the voltage time plot obtained on the oscilloscope during the tensile stressing of an aluminium specimen. It shows the generation of an electric field just at the instant of fracture. The voltage developed seems to be oscillatory in nature, marked by rapid damping. The diffused light in the vicinity of the horizontal time axis in the photograph is caused by the trace-blooming effects of the oscilloscope beam because of its excessive intensity. Similar curves were obtained for all metallic specimens (steel, aluminium, brass and zinc) but the amplitudes of the voltage generated were different for different materials. Figure 3 shows the tracings of the curves obtained on the storage oscilloscope for different materials, both qualitatively and quantitatively. The nature of the curve is independent of the orientation of the specimen. Expanding the time scale on the oscilloscope was not achieved because of the difficulty of synchronisation of the oscilloscope triggering to the instant of fracture. An expanded time scale would probably have exhibited the phenomenon more clearly.

Results with lead rods were of particular interest. Rapid fluctuations of electrical potential difference of the order of ±40 μV (peak value) ocurred as soon as the cross-sectional area of the lead specimen started reducing rapidly, instead of a first sharp peak value at the instant of fracture as in other cases. This may be because lead is very soft, plastic in nature and

Fig. 3 Tracings of plots obtained for: *a*, carbon steel; *b*, aluminium; *c*, brass; *d*, zinc. A, Denotes instant of fracture.

under uniaxial tension it suffers a rapid decrease in cross-sectional area leading to a point fracture. Moreover, the number of current carriers per atom based on a free electron model for lead is very high[2].

The twin phenomena of the generation of electric and magnetic fields at the instant of tensile fracture basically represent the electromagnetic effects of mechanical force fields in metals. Influencing parameters are likely to include: breaking stress, number and nature of current carriers per atom, electrical and magnetic structures of molecules of metals, mode of fracture, and crystal lattice structure. Furthermore, fracture phenomena are predominantly influenced by dislocations in dynamic state and energised shock waves. The observed electric and magnetic effects might be attributed to electron-dispersion analogous to the radiation of sound by dispersion of elastic vibrations of the crystal lattice as a result of dislocations[3].

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A new approach to improvement of yam *Dioscorea rotundata*

Efforts to improve the yam, *D. rotundata*, through hybridisation have been frustrated by the low rate of flowering, the very low rate of fruit setting and poor seed germination[1-3]. These constraints have confined the availability of genetic resources for selection to the small number of cultivars which have been vegetatively reproduced from time immemorial. This continued vegetative propagation and the lack of hybridisation have precluded the possibility of introduction of new genetic characteristics, resulting in a stagnation of yam improvement. To facilitate yam hybridisation, we have re-examined the problems of flowering and seed germination. We first reported[4] yam seed germination in November 1973, and Doku stated

that he had germinated yam seeds of *D. rotundata* (unpublished) Here we report the feasibility of germinating yam seeds, the rapid improvement of flowering, and the opportunity this development affords for expanding present germ plasm collections, thus facilitating yam improvement.

After a series of unsuccessful attempts, we obtained good germination in early 1973 when two important limitations for seed germination became evident[5]. First, the majority of the seeds were found to lack embryos or endosperms and second, they were found to possess a dormancy period of about 3 months after harvest. Having discovered these two limitations seeds that seemed to contain embryos were selected and stored at room temperature until the end of the dormancy period. Seeds were sown in Petri dishes in March 1973, and in about 3 weeks more than 50% of the seeds germinated. After germination and the appearance of the first leaf, some 600 seedlings were transplanted into small pots and a month later, were transferred to larger pots and grown in the greenhouse. Tuber formation began about a month after transplanting. Flowering of some plants began in July 1973 with all the flowering plants bearing staminate flowers. None of the plants had produced any pistillate flowers by plant maturity. The plants showed a great deal of variability with respect to leaf shape, size and colour, canopy structure, stem colour and shape, plant height, and tuber shape and size. Tubers were forced to maturity by gradual decrease in watering and were harvested in February and March 1974, at which time most of the tubers weighed more than 500 g, which contrasts markedly with the observation of Waitt[2], whose material took 2 yr to produce a 450 g tuber from seed.

On April 1, 1974, each tuber was divided into several 'seed' (vegetative) pieces and planted in the field. The purpose of this second planting was to facilitate maximum growth, flowering and fruiting under field conditions and to provide at maturity, seeds to produce plants of a second generation in 1975. Throughout the growing season, data were collected on all phases of plant growth characteristics with special attention to date and degree of flowering and plant sex. Male plants began to flower in early July while female plants began to flower in late July and early August.

It was observed that the percentage of flowering plants, particularly females, was greater in the population originating from seeds than in those originating from tuber cuttings obtained by continuous vegetative propagation (Table 1). The degree of flowering per plant had also increased. Whereas plants grown from tuber cuttings usually produce less than 200 pistillate flowers per plant, plants originating from seed produced from 500 to 11,000 pistillate flowers. Furthermore,

Table 1 Comparison of the degree of flowering and plant sex between plants of *D. rotundata* originated from continuous vegetative propagation and from seeds

	Flowering (%)	Non-flowering (%)	Flowering plants (%)		
			Male	Female	Monoecious
Plants from continuous vegetative propagation	47.1	52.9	69	31	0.0
Plants from tubers originating from seeds	72.3	27.7	53.6	41.7	4.7

Table 2 Seed germination and seedling establishment of eight Nigerian yam populations

Parent and source	No. of seeds tested	Germination (%)	No. of seedlings transferred to field	Seedling survival (%)
Ihobia (IITA)	330	90.0	89	43.8
Iwo (Kwara State)	2,175	99.3	1,385	88.2
Laoko (IITA)	50	—	9	100.0
Mixed (IITA)	360	—	37	100.0
Okunmodo (IITA)	260	92.4	58	34.5
Umudike (East Central State)	1,269	98.5	977	85.9
Unknown	230	—	12	100.0
Wild (IITA)	500	—	416	100.0
Total	5,147	95.1	2,983	95.7

Fig. 1 Degree of yam flowering and fruit set. *a*, Male plant; *b*, female plant at the fruiting stage.

4.7% of the plants derived from seeds, produced both staminate and pistillate flowers on the same plant (monoecious), which is an important departure from the usual production of either staminate or pistillate flowers on separate plants (Table 1). This quantitative and qualitative change in the flowering habit of plants of the first generation presumably resulted from our conscious selection for flowering through propagating plants from seeds. Since the low degree of flowering has been one of the major limiting factors in seed production, it is expected that after several generations the degree of flowering will increase to an extent such that it should be feasible to produce enough seeds to make yam propagation through seed a reality on a commercial basis. The increase in flowering and fruiting of plants produced from seed, did not reduce tuber yield. Tubers of up to 9 kg per plant were produced by plants from seed. It was notable that non-flowering plants yielded less than flowering plants and pistillate lines yielded more than staminate lines, thus suggesting that attention should be given to the former in selecting for high yields.

In a continued effort to increase our germ plasm collection by propagating plants from seeds, mature yam fruits were collected in October and November 1973 from eight sources in Nigeria. The seeds were germinated at the termination of the dormancy period as described earlier and the established seedlings were planted directly in the field. Seedling survival in the field was about 95% (Table 2), thus establishing the feasibility of planting yam seedlings directly in the field without the need for a pre-growth period in the greenhouse. Tremendous morphological differences exist between plants with regard to their canopy structure, leaf shape and size, climbing habit, stem colour and thorniness, date of flowering and abundance of flowers.

This study clearly demonstrates the feasibility of yam seed germination on a large scale and the possibility of greatly increasing the potential for improving genetical diversity and breeding resources. Whereas breeders have hitherto been limited in their selection schemes to the narrow genetic base in cultivars that have been under continuous vegetative propagation, it is now possible to select for desirable characters from almost unlimited numbers of new lines.

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Spawning pheromone in crown-of-thorns starfish

LARGE aggregations of the crown-of-thorns starfish *Acanthaster planci* L have destroyed a high proportion of the coral on certain Indo-Pacific reefs. We report that gamete release by one *A. planci* induces other ripe starfish to spawn; similar behaviour has been observed in certain other echinoderms[1,2]. During natural spawning a pheromone is released from the gonad, which synchronises spawning in neighbouring animals and also induces starfish movement towards the spawning individual. Using a Y maze to study chemoattraction in spawning starfish, we have attempted to isolate and characterise the active gonad component.

Several starfish (between 8 and 15) were maintained in a large Y-shaped tank which allowed an equal water flow through each of the two arms towards the base. *A. planci* normally avoids light and during the day remains in a covered area at the base, only moving into the uncovered arms at night. On six occasions, however, a single starfish became active during the mid-afternoon, and subsequently spawned, after which the remaining starfish emerged and several spawned (15 animals in 34 trials). Out of the 34 trials, 29 starfish moved towards the arm containing the spawning animal and five towards the control arm ($\chi^2 = 15.4; P < 0.001$).

When a starfish spawns in an aquarium, other starfish in the same tank or in tanks on the same filtration circuit, also frequently spawn; those that do not may, however, show behaviour typical of spawning. Spawning behaviour may also occur in response to l-methyl adenine injected at too low a dose to cause spawning itself (C. Huxley, personal communication). This 'spawning behaviour' consists of rapid movement with all tube feet active, and with the starfish partially inverted at the water surface; it probably corresponds to the climbing behaviour observed in spawning *A. planci* in the wild[3,4]. Similar behaviour has been observed in *Crossaster* when spawning in aquaria[5]. Daytime movement and spawning behaviour are thus more consistent responses to a spawning stimulus than actual spawning, presumably related to the physiological limitation on frequent spawning.

Daytime movement could be induced by feeding stimuli such as coral extract; thus, although induction of movement provided

Table 1 Response of *A. planci* to chemical stimuli

Substance added	% Movement	% Spawning behaviour	% Spawning	No. of trials
None	11	1	1	206
Whole gonad	77	22.5	13	31
Sperm-free solution	100	100	0	2
Sperm	87	73	0	15
Retentate	30	15	0	6
Boiled fraction	40	10	10	10
Zeocarb (*p*H 7)	27	4	0	26
Zeocarb NH₃ fraction	51	13	13	15
GSS	0	0	0	6
Glycine 10⁻⁶M	0	0	0	7

Observations were made during the afternoon every 15–30 min for 1 h before addition of material to the tanks and 3–7 h thereafter. Forty starfish were available for assays, two to three to a tank (50 × 60 × 25 cm). Movement was recorded if an individual starfish moved during three successive observations; 'spawning behaviour' required a single observation during the experiment. The number of trials is the number of starfish assayed with each type of material. Figures indicate the percentage of starfish responding. Whole gonad extracts were prepared by distilled water lysis of male or female gonads treated previously with GSS. Sperm solutions were obtained from tanks in which starfish had spawned spontaneously. Retentate, material remaining in a dialysis bag after dialysis, against 3 × 2 l of distilled water, of filtered whole gonad extract (usually 200 ml). Boiled fractions are gonad preparations or sperm solutions boiled for 40 min. Dialysate of whole gonad extract was passed through a 10-ml column of Zeocarb 225 cation exchange resin at *p*H 7. The material which failed to attach to the column is 'Zeocarb *p*H 7', and that which was eluted with 1 M NH₃ is 'Zeocarb NH₃ fraction'. The gonad extracts were assayed at a concentration of the total gonad from 1 starfish per 50 l.

a convenient assay, a putative spawning factor cannot be judged on this criterion alone. The active component seems to be present in the gonad (Table 1) but not firmly attached to the gametes themselves; it is dialysable and heat-stable. Kanatani[6] reported that neither gamete-shedding substance (GSS) from radial nerves nor 1-methyl adenine act as pheromones in *Asterias amurensis*, although both are intimately involved in the internal control of spawning. We confirm that GSS has no effect when added to the aquarium, but the starfish could detect 1-methyl adenine at 10⁻⁸–10⁻⁹ M when applied externally. All eight starfish tested extended their previously retracted tube feet, but only one starfish showed full spawning behaviour. No response was obtained, however, at concentrations up to 10⁻⁷ M, although the spawning season had ended by this time. The role of 1-methyl adenine has yet to be confirmed.

Ovary and testis extracts (data not shown) are equally rich sources of active spawning factor and in contrast to certain echinoids[1], there seems to be no differential sensitivity between males and females. We have no evidence for a strict periodicity in the responsiveness to extracts; however, those animals which had spawned recently or tended to spawn most frequently in the aquarium were more responsive than those which spawned rarely or not at all.

Whereas gonad development seems to be largely controlled by seasonal factors, the triggering stimulus for spawning in an aggregated population may be the release of gametes by neighbouring starfish. Spawning can occur in isolated individuals (D.H.B., N.J.H., and R.F.G.O., unpublished) but probably results in negligible fertilisation. In the case of mini-aggregations[7], however, in which up to 15 starfish may be clustered around a single coral head (such as a large table of *Acropora*), a pheromone capable of attracting starfish over a distance of a few metres and also synchronising gamete release, would greatly increase the success of spawning. It has already been suggested[8] that a large increase in spawning efficiency caused by aggregation formation may contribute to the instability of *A. planci* numbers and the occurrence of periodic population explosions.

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Production of verbenol pheromone by a bacterium isolated from bark beetles

THE aggregation pheromones of the bark beetle, *Ips paraconfusus* Lanier[1] are expelled in the faecal pellets of male beetles feeding on the phloem of *Pinus ponderosa*[2,3]. These substances, *cis*-verbenol, ipsenol, and ipsdienol[4], seem to originate in the hindgut[5] but the precise site of their biosynthesis has not been determined. In various species of *Ips* a connection has been convincingly demonstrated between the production of these pheromones in the hindgut and either ingestion of phloem or exposure of the beetles to host plant oleoresin[6]. More specifically, exposure of individuals of certain species of *Ips* to myrcene can result in an increased production of ipsenol and ipsdienol[7] and exposure of certain species of *Dendroctonus* to α–pinene results in an increased production of *cis*- and *trans*-verbenol[8–10] in the hindgut. It seems therefore that a precursor–product relationship exists between certain host plant substances such as α–pinene and myrcene and the three aggregation pheromones mentioned above.

A symbiotic relationship between an insect and a species of bacterium with respect to the production of the insect's sex pheromone, has been found in the New Zealand grass grub beetle[11] which carries a phenol-producing bacterium in the colleterial glands of the female. To our knowledge, however, the presence of microorganisms within the gut of an insect capable of transforming certain precursors to its pheromones has not been suggested.

Certain microorganisms can transform α–pinene into various substances including *cis*-verbenol and verbenone[12–15]. We therefore isolated a number of different microorganisms from the gut of adult male and female *I. paraconfusus* and determined their ability to transform α–pinene into *cis*- and *trans*-verbenol. All isolated species were grown aerobically in 100 ml of 3% yeast extract in a mineral salts medium[16] and shaken continuously. Good growth occurred in all cases within 24 h at which time 0.5 ml α–pinene was added and the flasks shaken for a further 24 h. Controls without either the microorganisms or α–pinene were run in the same manner. All flasks were extracted with ether, and, based on retention data of temperature-programmed gas chromatographic runs of the extracts (10% SP-1000, 60 °C to 200 °C at 10 °C min⁻¹), one organism in particular seemed to be producing the verbenols and this was identified as *Bacillus cereus*. This was isolated from both male and female beetles. Additional cultures of this organism were grown in 10 ml of the same medium, shaken for 24 h, and 0.1 ml α–pinene, purified by preparative gas chromatography, added for a further 24 h. Ether extracts of the cultures, and of

appropriate controls, were prepared and an aliquot of each extract analysed by gas chromatography on 10% EGS (140 °C) Peaks corresponding to both *cis-* and *trans*-verbenol were found in the culture extracts while none was detectable, even at high sensitivity, in the controls

The verbenols were purified from the remaining culture extracts by the consecutive separation, collection, and rechromatography of the appropriate peaks on four different stationary phases These were, in order of use, 10% Apiezon L (140 °C), 10% SP-1000 (140 °C), 10% EGS (140 °C), and 3% OV-225 (100 °C) Mass spectra of the purified substances eluting from the OV-225 column, at the same retention time as *cis-* and *trans*-verbenol, were recorded and the congruence of these spectra with those of standard spectra, also purified by gas chromatography, confirmed that both verbenol isomers had been synthesised from α-pinene by *B cereus* The yields of *cis-* and *trans*-verbenol were approximately 0 1% and 1% respectively of the added α-pinene Another major product of this transformation has been tentatively identified as myrtenol

α-Pinene   *trans*-Verbenol   *cis*-Verbenol

In spite of the numerous studies on the aggregation pheromones of *Ips*, the site of their synthesis has not been clearly defined Hughes[10] has argued that detection of the verbenols and other oxidation products in the haemolymph of the bark beetles, *D ponderosae* and *D valens*, after exposure to α- or β-pinene, established that their production occurs outside the alimentary canal It is assumed that these oxidation products are secreted into the hindgut and concentrated there by the reabsorption of water In contrast to this hypothesis, we consider that exposure of an individual beetle to the vapour of a particular monoterpene will saturate both the haemolymph and the gut contents with the monoterpene The oxidation of α- or β-pinene by microorganisms within the gut would then account for the greatly increased amounts of their oxidation products in this region and the detectable amounts in the haemolymph could occur either by diffusion out of the gut or by production of small amounts in the haemolymph, or by both processes

We have found that α-pinene, the most likely precursor of both the verbenols and myrtenol, is a major component of the monoterpenes present in the phloem of ponderosa pine Therefore, by either feeding or exposure, or both, this monoterpene would be present in the gut of male *I paraconfusus* during nuptial chamber excavation The occurrence of α-pinene throughout the gut of feeding *I calligraphus*[6] has been shown, and a number of *Dendroctonus* species produce increased amounts of the verbenols in their hindgut after exposure of individuals to α-pinene[8–10] These and other observations indicate that either ingestion of α-pinene, or intimate contact with α-pinene, or a combination of both of these conditions, is a prerequisite for the production of the verbenols by the various bark beetles It is obvious that under natural conditions of a beetle excavating a gallery both mechanisms would be operative *B cereus* present in the gut must come into contact with this monoterpene and presumably could bring about the oxidation of α-pinene to the verbenols Significantly, myrtenol is also known to be present in the gut of certain bark beetles after exposure to α-pinene[8,10]

A *Bacillus* sp capable of converting α-pinene to the verbenols has also been isolated from both males and females of *I grandicollis* and three species of *Dendroctonus* While our preliminary results do not prove conclusively that *B cereus* actually synthesises the verbenols from α-pinene in the hindgut, our data clearly indicate that this is a distinct possibility and substantiate the hypothesis that microorganisms may play a significant role in the synthesis of certain pheromones occurring

in the frass of these bark beetles The known differences in the quantitative production of the aggregation pheromones between males and females in these two genera remains enigmatic and is the subject of continuing investigations

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Stability of feasible predator-prey systems

Roberts[1] added a new dimension to the stability analysis of model ecosystems—feasibility, the requirement that equilibrium densities of species be positive Previous work[2–4] studied the stability of randomly constructed interaction matrices, *S*, which represented ecological interactions in the neighbourhood of an assumed feasible equilibrium point

Investigating the normal sort of quasi-linear ecological model, from which both equilibrium densities and stability can be determined, Roberts concluded that "almost all feasible systems are stable when the number of interacting species increases without limit"[1] Since real ecosystems are, *ipso facto*, feasible, this implies large ecosystems are more stable than small ecosystems—the tropics are more stable than the arctics This is the first instance where it is claimed that mathematics has supported the conventional wisdom of the natural historians diversity begets stability Mathematical analysis, summarised by May[4], has controverted this, yielding a new conventional wisdom stability permits diversity Roberts used the model,

$$dN_i/dt = N_i(r_i - \Sigma_j a_{ij}N_j), \quad i=1 \text{ to } m, \qquad (1)$$

in which he assumed $r_i = 1$, $a_{ii} = -1$ and $a_{ij} = \pm z$ where $+$ and $-$ are equally likely I have investigated the feasibility and stability of this model as a function of the interaction strength, z, and the number of species, m (Table 1) I have done the same for a modified version of this model in which $r_i = \pm 1$ and $a_{ii} = \pm 1$ where $+$ and $-$ are equally likely, and where a_{ij} are as before (Table 2)

Table 1 Feasible cases in 500 randomly constructed instances of Roberts' model The stable cases are indicated in the parentheses

No of species (m)	Strength of interaction (z)					
	0 2	0 4	0 8	1 6	3 2	6 4
2	500 (500)	500 (500)	500 (500)	133(0)	129 (0)	125 (0)
3	500 (500)	395 (395)	411 (411)	95 (20)	86 (12)	101 (16)
4	500 (500)	305 (305)	260 (257)	51 (3)	35 (5)	21 (4)
5	480 (480)	193 (191)	136 (127)	24 (3)	28 (5)	19 (3)
6	446 (446)	116 (114)	77 (63)	8 (1)	9 (1)	12 (2)
7	389 (389)	58 (53)	34 (32)	3 (0)	3 (0)	3 (1)
8	328 (328)	31 (29)	17 (11)	1 (0)	4 (2)	2 (1)
9	262 (262)	11 (11)	4 (3)	0 (0)	4 (0)	1 (1)
10	228 (228)	7 (5)	2 (2)	0 (0)	1 (1)	1 (0)

For both models, feasibility decreases with increasing m Non-feasibility corresponds reasonably to the competitive exclusion of one or more species (It can mathematically, but unreasonably, correspond to ecosystems whose biomass grows exponentially without limit) Therefore, both models predict greater instability, in the sense of increased frequency of competitive exclusion, in randomly constructed systems with greater numbers of species This is certainly no support for the conventional wisdom of the natural historians

The two tables indicate, moreover, that the stable percentage of the feasible cases behaves differently for the two models, increasing with m in Roberts' model, decreasing with m in the second model This means, at least, that Roberts' conclusion is fragile—not robustly insensitive to his assumptions And it suggests that his results may be artefactual

This is certainly the case Because he assumed r_i to be positive for all values of i, each of his species is autotrophic, that is, each can grow in the absence of all other species Alternatively, the species are heterotrophs and their energy supplies are implicitly included Therefore there is neither predation nor resource competition in his model, but only interference and facilitation (each equally likely) between species on a trophic level Mutual interference is reasonable[6], but the other interactions between pairs are evolutionarily absurd Why should the members of one species facilitate the members of a species that are interfering against them? Furthermore, mutual facilitation does not commonly occur between species on the same trophic level (interspecific bird flocks may be a counter example) Odum[6] states, "mutualism is most likely to occur between organisms of widely different requirements"

The second model does permit multiple trophic levels and predator–prey interactions Even though it permits some[f] absurdities (1 eats 2, who eats 3, who eats 1), it is clearly a more reasonable model of ecological interactions than the model Roberts chose The conclusions from the second model are consistent with the mathematical analysis of diversity and stability summarised by May[4]

One apparent difference exists between the second model and May's work stability increases with z in the second model ($P < 0.01$), whereas it decreases with interaction strength in May's work The interaction matrix, a_{ij}, of the second model

Table 2 Feasible cases in 500 randomly constructed instances of the second model The stable cases are indicated in the parentheses

No of species (m)	Strength of interaction (z)						Average percentage stable
	0 2	0 4	0 8	1 6	3 2	6 4	
2	119 (22)	122 (29)	120 (28)	117 (39)	109 (39)	132 (43)	28 3 ± 3 0
3	67 (8)	57 (8)	49 (5)	62 (14)	69 (17)	61 (19)	19 1 ± 3 3
4	30 (3)	35 (0)	33 (3)	29 (7)	33 (7)	29 (4)	13 0 ± 3 6
5	17 (1)	20 (4)	17 (1)	15 (3)	22 (4)	10 (1)	13 0 ± 2 9
6	5 (0)	7 (1)	12 (0)	7 (0)	11 (0)	3 (0)	2 38 ± 2 3
7	3 (0)	5 (0)	8 (2)	5 (0)	3 (0)	4 (2)	12 5 ± 8 5
8	1 (0)	4 (0)	1 (1)	3 (1)	1 (0)	1 (0)	19 0 ± 14 2
9	2 (0)	3 (0)	2 (0)	1 (0)	4 (0)	1 (0)	0
10	0 (0)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	0
Total stable cases	34	42	40	67	67	69	

; not, however, the stability matrix This is $S_{ij} = \overline{N_i}a_{ij}$, where $\overline{N_i}$ is the equilibrium density of species i ($\overline{N_i} = \Sigma_j a_{ij}^{-1}r_j$) for the second model, the average and median $\overline{N_i}$ decrease with z Biologically, the predators exploit the prey beyond the optimal yield, lowering the densities of all populations This is certainly the basis for the above noted discrepancy A deeper investigation into this phenomenon is in progress

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Influence of menstrual cycle on volunteering behaviour

RANDOM population sampling is rarely achieved in human research Rosenthal and Rosnow[1] have concluded that human behavioural science is largely the science of punctual college sophomore volunteers Volunteers typically have more education, higher occupational status, earlier birth position, lower chronological age, higher need for approval and lower authoritarianism than nonvolunteers[2-9] We report evidence here to suggest that the volunteering behaviour of college women varies with the stage of their menstrual cycle This implies that many experiments which report perceptual, cognitive or physiological response differences between the sexes may be biassed in systematic fashion by sampling error

In the initial experiment, women from two sections of a University of San Francisco child psychology class completed, in class, a questionnaire consisting of eight items on attitudes towards family planning, alcohol and drug use on campus, and peer-group and parental relationships Each respondent's age, social security number, religion, average menstrual cycle length, use of oral contraceptives and date of onset of the most recent menses were also obtained Social security numbers were used for data tabulation and the respondents were assured that the information would be kept confidential and used only for scientific purposes Two days later a male social psychologist, not associated with the original questionnaire, was introduced to the class and briefly described a research project for which he needed volunteers For one section, the proposed project required that the volunteers work alone on a learning task For the other it required that they work as a group in a decision-making task Sign-up sheets were subsequently passed around the class As in many psychology classes, such requests for volunteers were a common occurrence

To establish whether the stage of the menstrual cycle was related to volunteering, the cycle of each woman not taking oral contraceptives was divided into five phases on the basis of the questionnaire data The midpoint of the 'ovulatory phase' was calculated from the reported average cycle length by subtracting 14 d The 2 d on each side of this midpoint

were included in this phase, making it 5 d long The 'menstrual phase' was calculated as 5 d extending from the onset of menstrual bleeding, the 'preovulatory phase' made up the days falling between the menstrual and ovulatory phases, whereas the 'postovulatory phase' made up the first half of the period extending from the end of the ovulatory phase to the beginning of the next presumed menses The second half of this period was termed the 'premenstrual phase'

Since the type of experiment did not affect the frequency of volunteering, the data for both lecture sections were combined and are presented in Table 1a Most of the volunteers were in the ovulatory phase, whereas most of the nonvolunteers were in the postovulatory, premenstrual and menstrual phases ($\chi^2 = 22\ 47$, d f $= 4$, $P < 0\ 001$)

To establish whether this phenomenon was dependent on the sex of the individual seeking volunteers, we repeated the experiment a year later in a different class section using a female social psychologist In this case, the questionnaire data were obtained on the same day as the request for volunteers Table 1b shows that the results were essentially the same as in the previous experiment ($\chi^2 = 25\ 31$, d f $= 4$, $P < 0\ 001$)

Direct evidence for this phenomenon is difficult to find in the literature, since few investigators report the testing order of volunteers within the menstrual cycle In a recent study of taste preferences, Wright and Crow[10] divided the menstrual cycle of their volunteer subjects into five equal phases It is interesting that the number of subjects tested in the five phases (sequentially from menses) was 9, 18, 24, 25 and 17, respectively ($\chi^2 = 8\ 88$, d f $= 4$, $0\ 05 < P < 0\ 10$) Many behavioural studies reporting sex differences have assumed a random distribution of volunteering during the menstrual cycle because no relevant data were collected, and even studies examining menstrual cycle phenomena directly have made such an assumption For example, the authors of one study[11] reporting changes in visual sensitivity during the menstrual cycle stated "Because they were initiated into the test program as they volunteered, session order for the females was independent of the phase of the menstrual cycle" Our results suggest that this assumption is questionable and that test order should be counterbalanced so that systematic order effects are not confounded with endocrine influences

Several explanations of our findings seem plausible Some women, at least in Western cultures, undergo cyclic changes in emotions during the menstrual cycle Premenstrual and menstrual increases in tension, irritability, depression, anxiety and fatigue have been reported, as have increases in elation and activity near the time of ovulation[12-14] Such psychological and physiological changes may influence directly the tendency of women to volunteer for research It is possible that tendencies to respond to convert or overt classroom teacher and/or peer-group pressure are related to changes in 'response criteria' noted in signal detection analyses of perceptual phenomena during the menstrual cycle[15]

Regardless of the specific causal factors involved, our results suggest that many experiments which use female volunteers test a disproportionate number whose hormonal status is skewed from that of the general population of females The degree of skewing would depend on factors such as the length of time between the act of volunteering and actual participation in the experiment If such participation has followed soon after the time of volunteering, much of the literature on human females may be representative of punctual college sophomore

Table 1 Number of volunteers and nonvolunteers in each of the five phases of the menstrual cycle

Phase	Menstrual	Preovulatory	Ovulatory	Postovulatory	Premenstrual
a Male experimenter					
Volunteers	0	7	14	5	3
Nonvolunteers	7	4	1	12	5
b Female experimenter					
Volunteers	3	2	7	1	0
Nonvolunteers	23	4	1	14	4

volunteers who are probably not menstruating and are of ovulatory status

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Differential rates of cerebral maturation between sexes

TAYLOR and Ounsted[1,2] have proposed a model to account for the age of onset of developmental disorders and their relative incidence between the two sexes They propose that there is a period in the maturational continuum during which the child is particularly vulnerable to a particular disorder Males develop more slowly than females and they should therefore reach this "dangerous state" later, and take longer to pass through it It is therefore predicted for any developmental disorder, that males should show a later age of onset and a higher incidence than females[2], but that females are more likely to suffer seriously than males[1], (although this does not follow directly from the model) Evidence relevant to these predictions is reported here for congenital hydrocephalus

Medical records are available for 48 children who were referred to the Neurosurgical Unit at the Radcliffe Infirmary, Oxford, over the past 20 years, and who were diagnosed as suffering from congenital hydrocephalus In most cases there was no associated spina bifida cystica or other complex multiple congenital deformities For each patient, it was established whether surgical intervention had been necessary, or whether the hydrocephalus had arrested spontaneously (Table 1)

The series contained 33 males and 15 females This is equivalent to a sex ratio of 220 males for every 100 females, and differs significantly from equiprobability according to the binomial test ($z = 2$ 56, $P < 0$ 01, two-tailed test) The median age at which the boys were referred was 26 weeks, the median age at which the girls were referred was 7 weeks The Mann–Whitney U test demonstrated that the boys were significantly older when referred than the girls ($z = 1$ 69, $P < 0$ 05, one-tailed test)

In the case of 23 of the children (48%), the hydrocephalus

arrested spontaneously This compares closely with a figure o 46% reported by Laurence and Coates[3] from a much large series Considering boys and girls separately, it was found tha spontaneous arrest occurred in 17 (52%) of the boys, and in ((40%) of the girls Thus, the girls were more likely to require surgical intervention than the boys The difference is no statistically significant, however, according to a χ^2 tes which used the correction for continuity ($\chi^2 = 0$ 18, d f $= 1$ $P > 0$ 5) The median age at which a patient was referred if his hydrocephalus arrested spontaneously was 48 weeks, the median age at which he was referred if surgery was necessary was 17 weeks The difference between these two medians approaches statistical significance, according to a Mann–Whitney U test ($z = 1$ 82, $P < 0$ 1, two-tailed test) Both groups, however, showed the effect of sex on the age at which they were referred, described above

These results support the two principal hypotheses which can be derived from the model proposed by Taylor and Ounsted[2] Congenital hydrocephalus occurs in boys later and more often than it does in girls This constitutes further evidence for two generalisations in the epidemiology of disease females are susceptible earlier than males, yet males are more susceptible than females The model proposed by Taylor and Ounsted links these two generalisations by a single formal mechanism

If the necessity for surgical intervention can be taken as a sign of the severity of hydrocephalus, there is also some evidence for their assertion that diseases tend to occur more severely in females than in males This evidence is, however, not supported by statistics Nevertheless, the findings of this study suggest that research into developmental disorders may increase our understanding of the nature of sex differences and the processes of cerebral maturation

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Nude mice with normal thymus

MICE homozygous for the mutation, nude (*nu*) have been reported to have no thymus[1], although they retain a pre-lymphoid thymic rudiment[2] Here, I report the occurrence of individuals that are by external signs (including hairlessness) a nude mutation but possess an apparently normal thymus

The outbred nude stock in this laboratory has been repeatedly reinforced with +/*nu* heterozygotes and some fertile *nu*/*nu* homozygotes (Laboratory Animals Centre, Carshalton) The ensuing appearance of 'nudes with thymus' suggested that this phenotype originated from Carshalton, but the alternative could not be excluded that either a new mutation or some infiltration of a hairless gene might have happened in this laboratory Similar findings were communicated to me by Dr D Wakelin of the Wellcome Laboratories for Experimental Parasitology at the University of Glasgow, who had obtained his breeding stock from me A Carshalton origin, however, seemed to be confirmed when in November, 1974, I obtained from there a large group of nude mice aged about 3 months Out of over 60 autopsied so far there was one with an apparently normal thymus and one with a minute 'thymus' These tissues are being examined

Table 1 Number of patients referred, by sex and age at referral

	Age (months)							
	0	1	2	3–5	6–8	9–11	12–23	24 and over
Males	1	5	3	7	5	3	5	4
Females	5	4	0	0	1	1	2	2

In an attempt to test the hypothesis that a crossing over
ight have occurred between the gene for athymia and a
parate, so far unsuspected, gene for hairlessness, 48 young
ere obtained from matings of animals supplied by Dr Wakelin
id known to have given previously at least one nude mouse
ith a thymus Although such phenotypes again occurred, not
single animal was found with a normal coat but no thymus
everal animals with unusually small thymuses were observed,
ut the significance of this is problematical

Work is continuing on the above and alternative hypotheses,
ich as selection of modifiers under systems of husbandry
here, in outbred stocks, nudes that happen to be fertile are
ivoured as breeders[3] The present interim report becomes
ecessary because of the hazard the occasional nude mouse
nth a normal thymus constitutes for the experimenter It is
ow advisable to ensure that all nude mice are tested for
thymia at autopsy

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Nonspecific and specific immuno-suppression in tumour-bearing mice by soluble immune complexes

SEVERAL reports have concerned themselves with specific
suppression (blocking) of tumour-immune responses by serum
factors from tumour-bearing animals[1-3], and nonspecific
depression of T lymphocyte responses in tumour-bearing
mice has also been described[4,5] Evidence exists that specific
blocking of lymphocyte-mediated immunity occurs by means of
either free antigen[6] or antigen–antibody complexes[7] It has
now been suggested that nonspecific suppression of lymphocyte
responses could also occur by means of immune complexes[8],
if one assumes that both T and B lymphocytes have F_c re-
ceptors for the antibody region of such complexes There
is a growing body of evidence for F_c receptors of T cells[9,10]
We have investigated the possibility that the nonspecific
depression of the T lymphocyte phytohaemagglutinin (PHA)
response seen in BALB/c mice carrying tumours, induced by
Moloney sarcoma virus (MSV) in the progressive phase of
growth[4], is caused by similar factors to those which cause
specific blocking in this system Our results suggest that while
tumour-specific blocking can be mediated by free antigen,
as well as antigen–antibody complexes, nonspecific blocking
of T-cell responses is mediated only by the latter

The technique for stimulation of DNA synthesis as an assay
for T lymphocytes responding to PHA or MSV tumour antigen
is reported elsewhere[4] A ^{51}Cr assay which measures specific
cytotoxic T lymphocytes was also used, the targets being
derived from MSV-infected mouse fibroblasts passaged once
through young BALB/c mice[11] All cultures were performed in
Dulbecco's modified Eagles MEM, supplemented with 10%
foetal calf serum (DF_{10}) Cells capable of nonspecific suppres-
sion of immune responses were obtained from spleen cells
from MSV progressor animals by velocity sedimentation[4]
Such cells represent that fraction of the total spleen population
which sediments with velocity 4 8–6 0 mm h^{-1} Mouse sera
were obtained by cardiac puncture, the serum being heat-
inactivated (56 °C for 30 min) and stored at −20 °C until use
Antigen–antibody complexes were eluted from the kidneys of
normal and MSV-infected mice by the method of Oldstone and
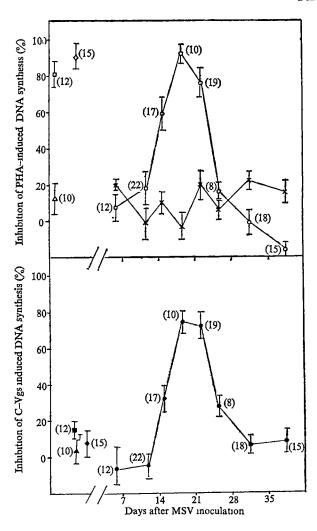
Fig. 1 Effect of tumour bearer and tumour regressor sera on
specific and nonspecific T-cell responses The assay used to test
for the suppression of the T lymphocyte response was to compare
the stimulation index

$$\frac{\text{c p m } ^3\text{H-thymidine incorporated in the presence of stimulus}}{\text{c p m } ^3\text{H-thymidine incorporated in the absence of stimulus}}$$

of 4×10^6 MSV regressor spleen cells (from mice given MSV 30 d
before) incubated in DF_{10} in the presence or absence of the
various sera For suppression of PHA responses (open symbols)
and LPS responses (crosses) the sera were used at a 1 10 final
concentration, for suppression of C-Vgs responses (closed
symbols) the sera were used at a 1 50 final concentration The
sera tested were ○, ●, ×, MSV inoculated animals, △, ▲,
normal mice, □, ■, polyoma tumour bearers, ◇, ◆, fibro-
sarcoma tumour bearers The number of mice used to provide
the pool of serum tested is shown in brackets beside the revelant
symbol All groups were set up in triplicate 95% confidence
limits are shown for each point The stimulation indices of the
MSV regressor cells used in the presence of DF_{10} alone were
PHA, 19±2 3, LPS, 23±4 1, C-Vgs, 6 2±1 2

Dixon[13] Rabbit anti-mouse immunoglobulin (anti-MIg)
columns were prepared as described previously[14] Absorption
of mouse sera on these columns (maximum capacity 2 mg
immunoglobulin) was performed at pH 7 0 in phosphate
buffered saline (PBS) Serum (0 5 ml) was washed on to the
column with 1 ml of PBS, the column left at room temperature
for 30 min, and the column was then washed with PBS until
the absorbance at 280 nm was less than 0 05 The absorbed
immunoglobulin was eluted at pH 2 6 with HCl glycine buffer.
The pH of the eluted immunoglobulin was adjusted to 7 0 with
phosphate buffer and both the unabsorbed effluent and the
eluted immunoglobulin were concentrated by vacuum dialysis
and dialysed against 200 volumes of PBS for 24 h

Figure 1 indicates the ability of serum taken from animals at
different times after MSV infection to inhibit the induction of
DNA synthesis by PHA, lipopolysaccharide (LPS), or MSV

Table 1 Effect of MSV sera or suppressor cells on response to PHA and C-Vgs

Velocity-sedimented suppressor cells added per culture	Final concentration of MSV serum present per culture	Stimulation index in response to	
		C-Vgs	PHA
None	None	5 5±1 1	24 ±3 6
2×10⁶		0 9±0 2	4 3±2 1
1×10⁶		1 1±0 3	6 2±2 1
0 2×10⁶		1 8±0 4	18 ±4 6
0 1×10⁶		3 2±0 5	19 ±3 7
	20%	0 8±0 3	2 6±1 3
	14%	0 9±0 2	6 3±2 1
	6%	1 2±0 2	10 5±3 2
	3%	2 9±0 5	18 ±4 1

2×10^6 spleen cells from MSV regressor animals (25 d after MSV inoculation) were cultured for 72 h in the presence of the stimulants shown (PHA, 1 μg ml⁻¹, C-Vgs, 5 μg ml⁻¹), with or without varying amounts of suppressor material All cultures were set up in triplicate The cultur were washed, pulsed for 12 h with 1 μCi of ³H-thymidine, and the TCA-precipitable material collected and counted in a liquid scintillatio counter The stimulation index was calculated as in the legend to Fig 1 95% confidence limits were calculated taking into account the variatio in both numerator and denominator

tumour-associated antigen (in this case a cell surface antigen with viral group specificity, C-Vgs) It is clear from these data that at around the time of tumour regression, serum factors appear which can block either of the T lymphocyte-mediated responses but not the B-cell response Moreover, the blockade of the C-Vgs response was mediated by lower concentrations of serum than were needed to cause nonspecific blocking The nonspecific blocking was seen with sera from other tumour-bearing mice (described elsewhere[15]), but the low concentration effect of serum on the C-Vgs induced enhanced DNA synthesis seems to be tumour specific[11] In our experiments normal mouse serum has never had a consistent suppressive effect on the splenic response to T or B cell mitogens (see Fig 1 and Table 2)

The correlation between the ability of sera taken from animals at different times after MSV infection to inhibit T lymphocyte responses in both a specific and a nonspecific manner agrees well with the simultaneous fall of each type of response in the spleen of MSV progressor animals[4] It is tempting to speculate that both the same serum factors and the same suppressor cell (already shown to be an immunoglobulin-bearing cell with sedimentation velocity 4 8–6 0 mm h⁻¹) cause these effects,

and that specific suppression is observed when the concentr. tion of suppressor is limiting Data to support this idea are show in Table 1 On dilution of sera or velocity-sedimented su pressor cells, the PHA response obtained from MSV regresso animals is restored before the C-Vgs response This is co sistent with the notion that the same cell type (or molecul produces each effect, the difference between seeing specific nonspecific inhibition being caused by the concentration cells (or molecules) needed to see each effect

In order to examine the possibility that immune comple cause both specific and nonspecific inhibition of T lymphocy function we have used an anti-MIg column in an attempt remove the blocking activity obtained from different source Three independent sources of blocking material have bee used (1) serum taken from mice 20 d after MSV inoculatio (Fig 1), (2) material eluted from the kidney basement mem brane of normal and the mice 20 d after MSV inoculation following a method previously described for obtaining immun complexes from such sources[13], (3) supernatants from 24 cultures of velocity-sedimented suppressor cells from MS' progressor animals, or their normal spleen cell equivalent Each supernatant was prepared from 1×10^8 cells, incubate

Table 2 Removal of blocking factors by an anti-immunoglobulin column

Source of blocking activity (concentration in PHA cultures, concentration with C-Vgs and in ⁵¹Cr assay)		Stimulation of DNA synthesis by		% Specific ⁵¹Cr release
		PHA	C-Vgs	
None		21 ±3 2	6 5±1 2	53±2 2
Normal serum (4mg ml⁻¹, 800 μg ml⁻¹)		22 ±2 6	6 7±1 1	52±1 9
20 d after MSV	Unfractionated (4 mg ml⁻¹, 800 μg ml⁻¹)	4 1±1 7	0 7±0 2	14±2 2
	Column effluent	16 ±2 7	2 8±0 3	40±1 7
	Column absorbed, acid-eluate	9 9±2 1	2 1±0 4	35±1 9
	Recombined fractions	6 8±2 4	1 3±0 4	15±1 5
Normal supernatant (400 μg ml⁻¹, 150 μg ml⁻¹)		22 ±1 7	6 7±1 3	52±2 1
Suppressor cell supernatant	Unfractionated (400 μg ml⁻¹, 150 μg ml⁻¹)	7 8±2 1	2 9±0 3	48±1 9
	Column effluent	19 ±2 6	5 8±0 5	50±2 2
	Column absorbed, acid-eluate	21 ±2 1	5 3±0 4	53±2 3
	Recombined fractions	17 ±3 2	6 2±0 3	51±1 9
Normal kidney extract (1 mg ml⁻¹, 300 μg ml⁻¹)		22 ±2 2	6 6±0 9	55±2 3
20 d after MSV	Unfractionated (1 mg ml⁻¹, 300 μg ml⁻¹)	5 1±1 6	1 3±0 2	17±1 9
	Column effluent	19 ±2 1	5 8±0 3	47±1 7
	Column absorbed, acid-eluate	18 ±3 1	6 4±0 9	51±2 0
	Recombined	8 2±3 3	3 1±0 3	28±2 2

Stimulation of DNA synthesis (using 4×10^6 regressor cells per culture) was as described in Table 1 and elsewhere[16] The final data are expresse as an arithmetic mean stimulation (compared with non-stimulated cells) with 95% confidence limits The ⁵¹Cr cytotoxic assay (final column) described in more detail elsewhere[11] Briefly, $7 5 \times 10^6$ regressor cells were incubated with 5×10^4 ⁵¹Cr lymphoma cells for 15 h at 37 °C, th final volume of the culture medium was 1 ml, and this medium also contained the blocking substances shown Percentage specific ⁵¹Cr releas was calculated as

$$\left(\frac{\text{c p m in supernatant with immune cells} - \text{spontaneous released c p m}}{\text{c p m in supernatant with detergent} - \text{spontaneous released c p m}}\right) \times 100$$

Normal cells routinely gave less than 0 5% specific release All cultures were set up in triplicate, the concentration of blocking factors in th tests used is shown in brackets thus (concentration with PHA as antigen, concentration with C-Vgs or lymphoma cells as antigen) Anti-MI column fractionated material was concentrated to the equivalent volume of the unfractionated materials

ı 5 ml serum-free Eagles' MEM with 5×10^{-3} M 2-mercapto-
thanol, and concentrated 30-fold by vacuum dialysis before
se

Each source of blocking material was tested with MSV
ıgressor cells in a DNA synthesis assay as in Table 1, and
ı a ^{51}Cr cytotoxicity test (with labelled MSV target cells)
'our fractions of each blocking material were analysed,
nabsorbed material, column effluent and acid-eluate, and the
ıtter two recombined at a 1 1 ratio The data for this experi-
ıent are shown in Table 2

Serum, kidney extract or cell culture supernatant derived
·om normal animals had no significant suppressive action
n the responses measured Whereas each of the unfractionated
locking materials derived from MSV-infected mice caused
oth anitgen-specific and nonspecific suppression of T-cell
ısponses, the effluent from an anti-MIg column (which we
resume contains free antigen) blocked only the antigen-
pecific responses (see effects of serum effluent on C-Vgs and
·HA induced DNA synthesis, and in the ^{51}Cr assay) The
olumn-absorbed, acid-eluted material, which could contain
xcess-free antibody and/or antigen-antibody complexes, again
·locked the antigen-specific responses, and often showed signi-
ıcant blocking of the nonspecific T cell response The best
uppression of the antigen-specific response, however, and often
he only suppression of the nonspecific response, was seen
·hen column-effluent and column-absorbed (acid-eluted)
naterial were recombined (giving antigen-antibody complexes,
ıerhaps in antigen excess) This is particularly well docu-
ıented for the MSV kidney extract, where only the recombined
naterial had any suppresive properties

We interpret these data as suggesting that optimum non-
pecific T cell blocking is mediated by antigen-antibody
omplexes, perhaps in antigen (column effluent) excess Antigen-
pecific T cell blockade is produced by lower concentrations
ıf such complexes, and, unlike nonspecific blocking, can also
ıe mediated by free antigen We feel that the failure to recover
ılocking activity from effluent, acid-eluate or recombined
naterial from the fractionated suppressor cell supernatant
·as a result of the low concentration of suppressor material
.pplied to the column in this case These findings do not
ıgree with an earlier report that in the MSV system in mice T
:ell cytotoxicity is not blockable by soluble tumour-associated
ıntigen (TAA) or progressor serum[3] The data do agree with the
eport that macrophage migration inhibition in this system,
ılso a T cell-mediated response[16], is inhibitable by progressor
era[17] A recent report indicates that T-cell-mediated cytotoxi-
:ıty in a rat Gross-virus induced lymphoma is also inhibitable
ıy soluble TAA or progressor sera[18]

It remains to be determined why T-cell cytotoxicity, which
s sensitive to lower concentrations of blocking factors in serum
ınd kidney extracts than the PHA response, is not blocked by
he suppressor cell fraction or culture supernatants which block
ıntigen-specific and PHA proliferative T-cell responses We are
nvestigating whether different TAA may be involved in T-cell
:ytotoxicity

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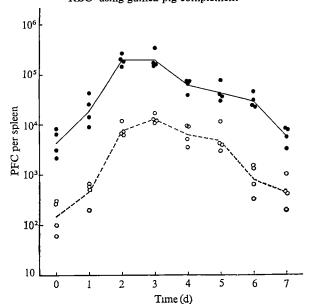
Active suppressor mechanism maintaining tolerance to some self components

NORMAL mice have in their lymphoid tissues a fairly large
number of cells making antibody to antigens present within
isologous erythrocytes[1] These plaque-forming cells (PFC) are
readily seen on red blood cells (RBC) treated with bromelain,
or other proteolytic enzymes[1] DeHeer and Edgington[2] have
recently described similar PFC in NZB mice

Certain facts suggest that this constant level of PFC in
normal mice is maintained as a balance between two opposing
forces, continual antigenic stimulation by self antigens on
the one hand, and some suppressor mechanism on the other
Constant stimulation seems likely since the antigens form
part of normal mouse RBC and probably become exposed
as red cells degenerate CBA mice usually have 2,000–7,000
PFC on bromelain-treated RBC by 3 weeks of age, and main-
tain these plaques for many months at about this level, which
is some 20 times higher than the background to heterologous
RBC (ref 1) Germ-free mice have similar numbers of plaques[1]
Specific suppression as a force opposing further increases in
the numbers of these PFC is suggested by two observations
First, injecting bromelain-treated isologous RBC at a variety
of dosage and routes gives little or no increase in PFC on
similar target cells (ref 1 and A J C, unpublished) Secondly,
cells potentially able to make antibody or antibody-forming
progeny to the internal RBC antigens are probably numerous,
since mitogen (bacterial lipopolysaccharide from *Escherichia
coli*) induces enormous numbers of plaques (Fig 1)

A way of testing for suppressive effects was suggested by
the experiments of Baker and colleagues[3,4], who showed,
using antilymphocyte serum (ALS), that suppressor T cells

Fig 1 Effect of an intraperitoneal injection of 50 µg of lipo-
polysaccharide on numbers of plaques to bromelain-treated
isologous RBC in mouse spleen ●, Plaques on treated mouse
RBC using rabbit complement, O, PFC against normal sheep
RBC using guinea pig complement

may diminish antibody responses to pneumococcal polysaccharide We made ALS by injecting rabbits intravenously with $2-10 \times 10^8$ mouse thymus cells on two to four occasions separated by intervals of 15–24 d Figure 2 shows that three injections of a total of 0 8 ml of this ALS increased the PFC numbers on bromelain-treated isologous RBC about 20-fold This is unlikely to have been a non-specific effect (that is, one caused by some general stimulation of immunocompetent cells), for several reasons The ALS had little or no effect on background plaques to sheep RBC (Fig 2), in sharp contrast to the stimulation of the anti-sheep response by mitogen (Fig 1) Normal rabbit serum had a much smaller stimulatory effect (Fig 2) In control experiments it was shown, first, that most of the activity of the ALS could be removed by absorbtion with mouse thymus cells, second, that the same pool of ALS decreased a response to injected sheep RBC about tenfold, and, third, by analogy with Baker's experiments[4], that the PFC number in nude mice was not increased by ALS injection, whereas that of their normal littermates was increased severalfold
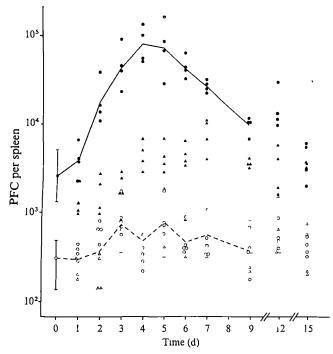
Fig. 2 CBA mice were injected with 0 2 ml ALS intraperitoneally and 0 2 ml subcutaneously on day 0 On days 2 and 4, they received 0 1 ml in each site Control animals received serum from a normal rabbit ●, ALS injected, PFC assayed on bromelain-treated mouse RBC ○, ALS injected, PFC on sheep RBC ▲, Normal rabbit serum injected, assayed on bromelain-treated mouse RBC △, Normal rabbit serum injected, PFC on sheep RBC The day 0 points show geometric means and range of values in 12 uninjected mice tested on three different days

The simplest explanation for these results is that ALS promotes the autoantibody response by removing suppressor cells The suppressors may be T cells, although the evidence is not conclusive This example of suppressor tolerance to a group of self antigens in normal mice has obvious similarities to the active suppression, in young NZB mice, of responses to their own red cells[5,6]

It is important to determine the scope of this kind of mechanism by investigating a number of different model systems, since if tolerance to many self components, as well as to many foreign antigens[7], is maintained by active suppression of potentially reactive cells, a completely new view of the workings of the immune system would emerge In extreme form, this may be stated as follows All antigen-reactive cells bind some self components to varying extents, and all are subject

to some degree of continuous suppression (this is not to dei that deletion of some clones may occur if they are faced wii overwhelming amounts of a soluble self antigen which the bind avidly) The success of a foreign antigen in provoking specific immune response depends on its ability to distui this balanced state, with antigens close to self being most liab to suppression, and therefore least immunogenic During ar immune response, cells making entirely new antibody spec ficities are rapidly generated[8,9], and these new variants ai also liable to suppression, so that only those which mo successfully avoid the suppression mechanism are seen Coi stant stimulation with a foreign antigen leads to its beir treated like self[10] more, and perhaps new kinds of, suppresso cells develop, and a state of greatly reduced responsiveness t that antigen eventually supervenes

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Separation of cells involved in phytohaemagglutinin-induced mitogenesis and cytotoxicity

LYMPHOCYTE mitogens such as phytohaemagglutinin (PHA) ma; induce lymphocytes to mitogenesis (DNA synthesis) an cytotoxicity (lysis of target cells) Although there is evidenc that these two responses are independent of each other i remains to be determined whether different lymphocyte sub populations are involved[1,2] It has been claimed that PHA is a selective T-cell mitogen but some recent work argues agains such precise specificity[3] There is also evidence suggesting that PHA may induce T cells to become cytotoxic but, since non-T cells may be cytotoxic under some conditions, the situation remains to be clarified[1,2,4–8]

In the course of investigation of the lymphocyte function o patients with immunological deficiencies, we have been im pressed by the frequent absence of PHA-induced mitogenesi; when PHA-induced cytotoxicity seems to be normal The reverse situation also occurs These observations suggested tha different lymphocyte subpopulations might be involved ir mitogenesis and cytotoxicity We report here evidence which shows that the cells involved may be separated by rosette sedimentation

Between 50 and 100 ml of heparinised blood were collected from seven normal subjects and centrifuged on Isopaque–Ficol gradients[9] with a yield of 100–200 mononuclear cells per individual Polymorph contamination was less than 5% whereas the percentage of monocytes was variable The separated mononuclear cells were then incubated with sheep red blood cells (SRBC) so as to allow the formation on non-immune T-cell E rosettes[10] The mononuclear–SRBC mixture was then layered on to Isopaque–Ficol and centrifuged as for the initial separation The cells collected from the interface were found to contain less than 5% E rosetting cells and from 34–61% B cells as indicated by the presence of readily demon-
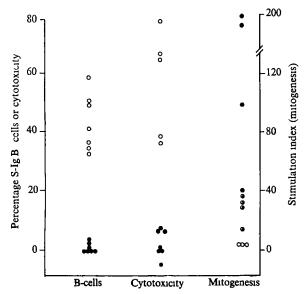
Fig. 1 The effect of rosette sedimentation on PHA-induced cytotoxicity, PHA-induced mitogenesis and B cells ○, Cells from the interface, ●, cells from the pellet, ◑, mononuclear cells before rosette sedimentation Stimulation index refers to counts after PHA stimulation divided by the resting level

trable surface immunoglobulin (S-Ig)[10] In contrast less than % and generally less than 1% of lymphocytes in the pellet were B cells with S-Ig Thus the interface population consisted f B cells with some "null" lymphocytes (non-E-rosetting, non--Ig) and some monocytes but very few E-rosetting T cells whereas the pellet population consisted of essentially pure T ells

These two populations were then compared in terms of PHA-induced mitogenesis and cytotoxicity The former was estimated by measuring the tritiated thymidine uptake after incubating 0^6 lymphocytes with 5 μl PHA-P (Difco) for 3 d Cytotoxicity was determined by counting ^{51}Cr release from 10^5 labelled chicken red blood cells after 18 h incubation with $2 5 \times 10^6$ lymphocytes and 10 μl of PHA[11] Contaminating SRBC were emoved from the pellet by lysis with 0 83% NH₄Cl–Tris-HCl pH 7 65) although in some experiments equivalent numbers of SRBC were added to the interface population

Results are summarised in Fig 1 There was a gross reduction in the PHA-induced cytotoxicity of the pellet population in relation to the interface population The reverse situation was seen with respect to mitogenesis induced by PHA The pellet population showed high stimulation indices relative to either the interface or the starting mononuclear population before rosette sedimentation

These data show that different subpopulations are involved in the mitogenic and cytotoxic responses to PHA It could be argued that the mitogenic response involves only E-rosetting T cells, but in other experiments we have found that there can be some stimulation of the interface populations and interpretation may be complicated by the fact that resting DNA synthesis is higher in the interface than the pellet population[12] For these reasons we cannot conclude that PHA mitogenesis is an absolutely specific T-cell marker but it would seem to be far more so than some others have suggested[3] E-rosetting T cells seem to contribute very little, if at all to PHA-induced cytotoxicity Whether B cells, non-E rosetting T cells, "null" lymphocytes or even monocytes are responsible for the cytotoxicity seen after incubation with PHA cannot be determined from the present study But we believe that our data clearly indicate that PHA-induced mitogenesis and cytotoxicity should be considered as representing different functions and populations when assessing patients with immunodeficiency states

Arthritis and Rheumatism Foundation, Muscular Dystrophy Associations of America and the Australian Kidney Foundation *Note added in proof* Work in progress (P J Z , G Grimsley and R L D , unpublished) suggests a major role for monocytes in PHA-induced cytotoxicity against RBC

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Sertoli cell secretory function after hypophysectomy

Testicular androgen is the principal stimulus for spermatogenesis[1] Androgens alone are capable of maintaining spermatogenesis in hypophysectomised rats when treatment is begun immediately following hypophysectomy[2] Following posthypophysectomy regression of the seminiferous epithelium, however, neither luteinising hormone (LH) nor androgen given alone is capable of initiating spermatogenesis when the same dose and time schedule is used for the treatment Follicle stimulating hormone (FSH) has to be given together with LH or androgen to effectively restore spermatogenic activity[1] This difference between main-
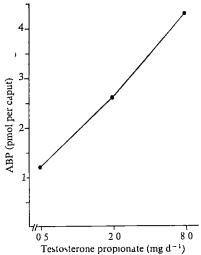
Fig 1 Maintenance of ABP production by testosterone propionate (TP) in hypophysectomised rats Male Sprague–Dawley rats in groups of four were hypophysectomised at 28 d of age and treated with various doses of testosterone propionate 0 5–8 mg d⁻¹ in sesame oil for 9 d, starting immediately after hypophysectomy The animals were then killed on day 10, testis and capita epididymides weighed and homogenised in four volumes of buffer (50 mM Tris-HCl, pH 7 4, containing 1 mM EDTA) and centrifuged at 105 000*g* for 1 h The supernatants were diluted with equal volumes of the same buffer containing 20% glycerol and 60 nM ³H-DHT Concentrations of ABP was then examined by steady state polyacrylamide gel electrophoresis (SS–PAGE) as previously described[11]

tenance (treatment started immediately after hypophysectomy) and initiation (treatment started after regression of the germinal epithelium) of spermatogenesis, has been known for more than 30 yr (ref 3), but no data have been provided which could explain this phenomenon

We have shown that one action of FSH in the seminiferous tubules is to induce the production of a specific androgen binding protein (ABP) by the Sertoli cells[4,5] This protein is secreted through the rete testis fluid and concentrated in the caput epididymis[6] We believe that the effect of FSH, mediated through ABP, is to amplify the testosterone stimulus to androgen-sensitive cells of the germinal epithelium Since ABP is produced in the Sertoli cell[7-9], we have suggested that this component can be used as a specific marker of Sertoli cell secretory function[10] We report here that androgen alone is capable of maintaining Sertoli cell secretory function in immature hypophysecto-
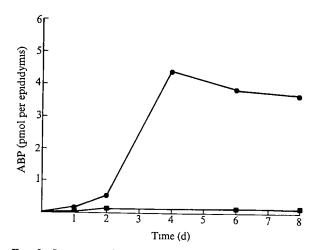
Fig. 2 Initiation of ABP production by FSH but not by testosterone propionate in hypophysectomised rats Rats (groups of four) were hypophysectomised at 28 d of age, and their testes allowed to regress for 10 d before testosterone propionate treatment Since 0 5 mg testosterone propionate daily was sufficient to maintain ABP in the capita epididymides for 10 d after hypophysectomy, animals were now treated with ten times this dose (■, 5 mg testosterone propionate daily) from 1–8 d starting at day 11 after hypophysectomy ABP was measured in capita epididymides using SS–PAGE as above Other groups of animals, hypophysectomised simultaneously, were injected with FSH alone (●, 250 μg NIH–FSH–S₁₀ per day) FSH was dissolved in saline containing 0 1% bovine serum albumin and 0 25 ml was injected subcutaneously twice daily (125 μg dose) TP was dissolved in sesame oil and 0 1 ml was injected intramuscularly once daily

mised rats, but is not able to restore the secretory activity of the Sertoli cell after posthypophysectomy regression

Figure 1 shows that androgen alone given to hypophysectomised animals is capable of maintaining Sertoli cell secretory function as measured by ABP levels in capita epididymides Increasing doses of testosterone propionate gave a dose-dependent increase in the amount of ABP per caput (Fig 1) as well as in testis weight (not shown) In the control animals (injected with oil only) no detectable ABP was present in the capita epididymides Figure 2 shows that androgen alone is not capable of stimulating ABP production by the Sertoli cell within 8 d of treatment Treatment with FSH, however, causes a rapid increase in ABP levels in the capita epididymides

We conclude, therefore, that the Sertoli cell is responsive to androgens as well as to FSH (refs 4–6 and 12–14) Following regression of the cells for 10 d without hormone stimulation (hypophysectomy), however, FSH is required for full restoration of the secretory capacity These studies also demonstrate the usefulness of ABP as a specific parameter of Sertoli cell secretory function

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Occurrence of synapses in peripheral sensory nerves of arachnids

RECEPTOR cells of arthropods are presumed to send their axons directly into the central nervous system[1,2] Second order fibres supposedly do not occur in the periphery, an thus the first synaptic connections ought to lie within the ganglionic neuropiles We report, however, that synapse do occur regularly in peripheral sensory nerves of at leas two arachnid orders, whip spiders (Amblypygi) and harvest men (Opiliones)

The first legs of whip spiders are modified appendage which are extremely long (20–25 cm) and slender (0 2 mn diameter) The tarsi are subdivided into about 100 segment and bear thousands of multi-innervated hair sensilla[3] A there are no muscles within the tarsus, the two tarsal nerve consist of afferent fibres only (with the possible exceptio of some efferent fibres supplying epidermal glands)

Tarsi of the first legs of *Admetus pumilio* (Amblypygi were prepared for electron microscopy and transverse sec tions at various segments (2, 4, 6, 8, 10, 13, 15, 17 and 60 were examined Synapses were observed in most cros sections of the two tarsal nerves and occurred only on on or a few postsynaptic fibres (Fig 1a), which are alway located at the periphery of the nerve Thus, the synapse are restricted to a very small area, while the surrounding thousands of axons comprising the leg nerve do not exhibi any junctional specialisation

The postsynaptic fibre is relatively large Short latera extensions and the presence of ribosomes and many mito chondria suggest a branching dendrite The presynapti elements may form rather conventional synapses (Fig 1a or 'dyad' synapses[4-9] (Fig 1d) In the former case, there 1
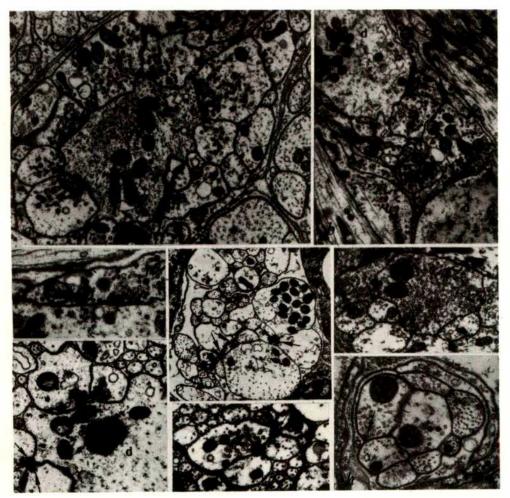
Fig. 1 *a–e*, *Admetus pumilio* (tarsus of leg 1). *a*, Part of a cross section from a tarsal nerve showing one relatively large dendrite (d), contacted by about ten presynaptic endings (×15,400). *b*, Para-sagittal section corresponding to Fig. 1*a*. The synapses are not evenly distributed along the long axis of the dendrite (d) but form clusters at regular intervals. Most synapses (arrows) contain round synaptic vesicles and presynaptic dense projections. Some terminals exhibit flattened synaptic vesicles (asterisk) (×10,500). *c*, Detail of a longitudinally sectioned terminal. The contact zone is delineated by a widened synaptic cleft. Several dense projections, a cluster of synaptic vesicles and few dense-core vesicles (arrow head) are typical for the presynaptic side. The postsynaptic side shows very little, if any, membrane thickening (compare with *a*) (×18,350). *d*, Part of a cross section of a tarsal nerve. Two presynaptic fibres form dyad synapses with a large dendrite (d). Another dyad synapse is indicated by arrows (×22,800). *e*, A bundle of intra-epidermal nerve fibres in cross section. Short extensions of two large fibres receive dyad synapses (arrows) (×9,800). *f–g*, *Phalangium opilio* (tarsus of leg 2). *f*, Cross section of a tarsal nerve showing typical dyad synapses. The small fibre on the right (long arrow) forms perhaps a reciprocal synapse with the large fibre (short arrow) (×28,000). *g*, Intra-epidermal nerve fibre, densely filled with synaptic vesicles, makes dyad synapses (arrows) with several adjacent fibres (×15,700). *h*, *Araneus diadematus* (tarsus of leg 1). Eight intra-epidermal nerve fibres in cross section. One fibre (arrow) exhibits synaptic vesicles and a dense plaque opposite the angle between the adjacent nerve fibre and a glial cell (g) (×17,500).

high concentration of synaptic vesicles and a distinct synaptic cleft, yet little membrane thickening. The presynaptic side often has dense projections (Fig. 1*c*) but the postsynaptic side is only slightly thickened, if at all. The dyad synapse always involves three nerve fibres, usually two presynaptic axons and one postsynaptic dendrite. Both axon terminals have an electron-dense plaque on the cytoplasmic side of the membrane surrounded by a small cluster of synaptic vesicles. The typical arrangement of dyad synapses is shown in Fig. 1*d*.

Longitudinal sections show that the synapses do not cover the entire dendritic surface, but form clusters (Fig. 1*b*) at intervals of 6–8 μm. *En passant* synapses are commonly seen, which means that one presynaptic fibre often makes several synaptic contacts. Additional small synapses may be found in close proximity to the relatively large dendrites. From serial sections it seems that these contact dendritic extensions of the large fibre.

Synapses were also observed outside the tarsal nerves, within intra-epidermal fibre bundles (Fig. 1*e*). These also form either conventional or dyad synapses. In the latter case, several presynaptic fibres converge on a larger postsynaptic fibre.

The legs of harvest-men are especially long, and an investigation of the tarsal leg nerves in *Phalangium opilio* also revealed synapses, mostly of the dyad type (Fig. 1*f*) and seemingly not restricted to large postsynaptic fibres. One presynaptic axon often forms a dyad with two small postsynaptic fibres. This then represents a divergent synapse rather than the convergent type found in the whip spider. Definite synaptic contacts were also seen in intra-epidermal nerve fibre bundles (Fig. 1*g*).

A brief survey of the tarsus in a web spider (*Araneus diadematus*, Araneae) revealed synapses in the leg nerves as well as within intra-epidermal fibre bundles (Fig. 1*h*). These are, however, much less conspicuous and seemingly rarer than in the whip spider or harvest-man. Contacts similar to those shown in Fig. 1*h* have been reported in insect gustatory organs[10], and have been interpreted as efferent and assigned a 'neurosecretomotor' function. This is also a possible explanation for the intra-epidermal synapses found in arachnids, but it could hardly be applied to the synapses within the leg nerves, because there are no effector organs except for some gland cells.

How can the presence of numerous, often considerably localised, synapses in sensory nerves of arachnid legs be

interpreted? At present, the origins of pre- and postsynaptic elements cannot be clearly determined and no definite answer can be provided. It seems likely, however, that the large dendrites belong to interneurones which receive the input of many primary receptors. At least in the case of the whip spider, the system could be comparable with the giant fibre system found in many invertebrates[11-15]— except, of course, that the giant fibres lie within the central nervous system. In the whip spider the large fibres which receive synapses attain a diameter of 10 μm (proximal tarsal level), whereas the majority of sensory axons measure 0.2 μm or less. The large fibre diameter could provide a greater conduction velocity for nerve impulses; this may be an important feature, if one considers that most receptors are more than 20 cm away from the central nervous system. An additional advantage would be a reduction in the high number (10^4–10^5) of primary receptor axons.

From the large number of synapses occurring in peripheral sensory nerves, it must be concluded that some integration is taking place at the periphery of the arachnid nervous system. This may set the arachnids aside from insects, in which the first site of sensory information processing is believed to lie within the central nervous system[2].

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Neurological changes in fruit bats deficient in vitamin B$_{12}$

HAEMATOLOGICAL and neurological sequelae are the two major effects of vitamin B$_{12}$ deficiency in man. In the haemopoietic system, megaloblastic change leading to anaemia seems to be caused by deranged DNA synthesis[1]. The cause of central nervous system demyelination, however, is unknown and progress in this area has been hampered by the lack of a suitable experimental animal model. We report that Egyptian fruit bats (*Rousettus aegyptiacus*), made vitamin B$_{12}$ deficient in captivity, develop neurological changes similar to those observed in human subacute combined degeneration of the spinal cord.

Previous attempts to find a suitable animal model for vitamin B$_{12}$ deficiency have proved difficult for several reasons. Herbivores obtain vitamin B$_{12}$ either by coprophagy[2] or, in the case of ruminants, from resident microorganisms in their upper gastrointestinal tract[3]. Consequently, it has been necessary to impose a variety of artificial procedures, such as the use of synthetic diets[4], the prevention of coprophagy[5] and antibiotics to eliminate gastrointestinal flora[6], to produce vitamin B$_{12}$ deficiency in animals. Furthermore, animals made vitamin B$_{12}$ deficient by these procedures do not develop consistent haematological or neurological changes. The most convincing evidence of neurological change in vitamin B$_{12}$ deficient animals was reported in primates which developed
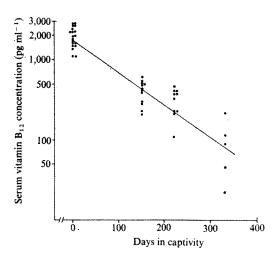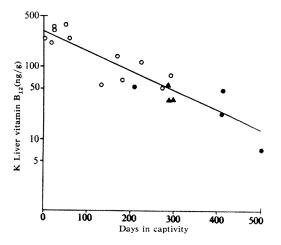
Fig. 1 Serum vitamin B$_{12}$ concentrations (log scale) at various times in 19 bats maintained on an all-fruit diet. The fall was exponential and highly significant after 330 d (t_{22} = 14.8, P < 0.001). The formula for the regression line was: $\log y = -0.0041 x + 3.245$ and the calculated turnover rate [$(\ln 2/t_{\frac{1}{2}}) \times 100$] was 0.93% d^{-1}.

'cage paralysis' on a vegetarian diet[7], but two recent studies failed to confirm these findings.

R. aegyptiacus (suborder Megachiroptera) is widely distribute in tropical and semitropical parts of the Old World. Th species has been successfully kept in European zoos since th last century[10], and its natural lifespan has been estimated be 15–20 yr[11]. We selected this species because its natural di consists entirely of fruit and flowers[10]. Also, because of the habit of foraging on fruit trees remote from the caves in whi they roost, faecal contamination of the diet is unlikely, ar furthermore, coprophagy has not been observed. Although is not known how these bats normally obtain their dieta supply of vitamin B$_{12}$, it is presumed that they may do so eith by inadvertent ingestion of fruit pests, or by drinking stagna water which may contain up to 1.0 ng vitamin B$_{12}$ ml^{-1} (ref. 12 We excluded these potential sources of the vitamin in captivi without otherwise altering the bat's natural diet.

Adult male bats captured in the North-eastern Transva were maintained on a pest-free all-fruit diet consisting washed and peeled bananas, papayas, pears and orang fed *ad libitum*. Bats were supplied with clean tap water dai and their cage floor was kept clean. Blood samples we

Fig. 2 Liver vitamin B$_{12}$ concentration (log scale) in 19 bats which were killed at various times while on an all-fruit diet. The fall was exponential and highly significant (t_{17} = 24.6, P < 0.001). The formula for the regression line was: $\log y = -0.0029 x + 3.061$ and the calculated turnover rate was 0.63% d^{-1}. Presence or absence of neurological changes shown by: ●, functional and structural abnormalities; ▲, functional, but no structural abnormality; ○, normal.

obtained by direct cardiac puncture for blood counts and measurement of the serum vitamin B_{12} concentration using a radioisotope dilution method[13]. Liver vitamin B_{12} concentration was measured by a modification of the serum method. Stained slides were prepared from rib bone marrow. Spinal cords were removed from freshly killed animals, sectioned and stained with haematoxylin and eosin, phosphotungstic acid haematoxylin, the Kluver–Barrera stain for myelin, Bielschowsky stain for axons and oil red O for lipids.

The serum vitamin B_{12} concentration (mean $\pm$ s.e.) in 19 bats fed this diet fell significantly from 1964.0 ± 121.01 pg ml^{-1} at the time of capture, to 98.6 ± 34.88 pg ml^{-1} in five surviving bats after 330 d (Fig. 1). The liver vitamin B_{12} concentration measured in bats killed at various times also fell significantly (Fig. 2). Over this period, there was no fall in the haemoglobin level, haematocrit or red cell count, nor any alteration in the calculated red cell indices. But the leukocyte count (mean $\pm$ s.e.) in 19 bats fell significantly from 19.5 ± 1.11 to 4.9 ± 0.38 thousand μl^{-1} in 12 surviving bats after 130 d ($t_{29} = 12.5$, $P < 0.001$). This fall reflected a decrease in both granulocytes and non-granulocytes, but no further significant change was observed beyond 130 d. Injection of 200 ng vitamin B_{12} per week in two bats resulted in a return of the leukocyte count to initial values. There was no increase in polymorphonuclear leukocyte lobe count, nor was there bone marrow evidence of megaloblastic change in either myeloid or erythroid precursors.
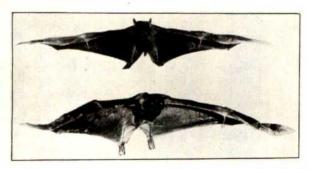
Fig. 3 Single representative frames of (*a*) normal and (*b*) deficient bats, taken during middle of downstroke. Three major functional alterations occur in the flight pattern: Gross irregularities of the surface of the dactylopatagium result in massive turbulence and partial stalling; the irregular surface of the tip negates the propulsive force normally arising from the downward twist of this region; slackening of the trailing edge (normally held taut between foot and fifth digit) increases drag.

After 200 d, as bats became progressively more vitamin B_{12} deficient (Fig. 2) seven of the ten surviving animals developed ataxia, manifested by difficulty in climbing. This seemed to result from a loss of reflex proprioceptive sensation in the lower extremities, as bats were unable to disengage their claws from the wire mesh during climbing.

Changes in the normal flying cycle were also noted, and were analysed by high speed cine and still photography. The most obvious abnormalities occurred during the downstroke, when the dactylopatagia were crumpled and irregular compared with the normally smooth, fully extended wing surface (Fig. 3). Full separation of the phalanges was lost, resulting in laxity of the individual membranes. At the beginning of the downstroke, when usually the phalanges are fully extended and radially deviated, those of the deficient bats trailed in the slipstream. The relatively coarser control of the shoulder, elbow and thumb was little affected but the weakened fifth digit led to slackness of the trailing edge of the plagiopatagium. The upstroke, being partly a passive phenomenon and requiring little precise aerodynamic control of the distal wing, was minimally affected.

Histological sections of the spinal cords of bats killed after 200 d and stained for myelin, showed patchy spongiose change
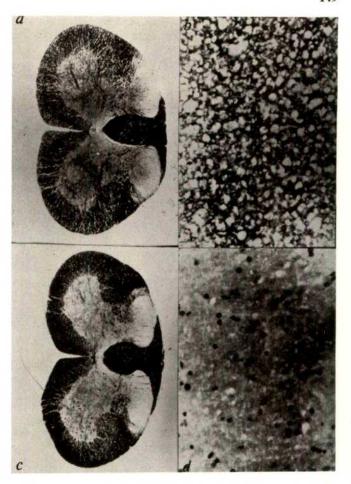
Fig. 4 Histological sections of the spinal cord taken from the upper thoracic region of fruit bats. Sections stained with Kluver–Barrera stain for myelin. *a*, Low power view ($\times$ 14) of section from a vitamin B_{12} deficient bat (serum vitamin B_{12} concentration 108 pg ml^{-1}; liver vitamin B_{12} concentration 54 ng g^{-1}) obtained 210 d after receiving an all-fruit vitamin B_{12} deficient diet. The white matter shows patchy spongiose change affecting mainly the lateral and ventrolateral columns. *b*, High power view ($\times$ 259) of the white matter from the same section (deficient bat). *c*, Low power view ($\times$ 14) of section from a normal bat (serum vitamin B_{12} concentration 2,340 pg ml^{-1}; liver vitamin B_{12} concentration 320 ng g^{-1}). *d*, High power view ($\times$ 259) of the white matter from the same section (normal bat). The section from the normal bat does not show the patchy spongiose change seen in the spinal cord of the vitamin B_{12} depleted animal.

in the white matter of the lower cervical and upper thoracic region, mainly affecting the lateral and ventrolateral columns and suggestive of early demyelination (Fig. 4). The changes were observed in four out of five bats which had developed evidence of functional neurological impairment; their liver vitamin B_{12} concentrations are shown in Fig. 2. The lesions are similar to those lesions found in vitamin B_{12} deficient humans except that histological changes in man affect mainly the dorsal and lateral columns[14]. There was no evidence of reactive astrocytosis or gliosis, and the stain for axons showed no differences compared with normal cords. No lipid-containing phagocytes were observed on an oil red O stain. Bats given 200 ng cyanocobalamin per week by intramuscular injection did not develop any neurological changes, but cyanocobalamin supplements failed to reverse functional changes once they had appeared.

In summary, we have shown that the fruit bat can be rapidly depleted of vitamin B_{12} when kept on a diet similar to its natural one and that fruit bats may be used as an experimental model to study the neurological changes produced by vitamin B_{12} deficiency in the absence of anaemia. Further studies are in progress on the nature of these neurological changes.

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Evidence for the B_{12}-dependent enzyme ethanolamine deaminase in *Salmonella*

THE enteric bacteria *Escherichia coli* and *Salmonella typhimurium*, like man, do not synthesise significant amounts of cobalamin (B_{12}) compounds and thus depend on exogenous vitamin B_{12} for their B_{12}-dependent enzymes[1]. In *E. coli* and *S. typhimurium* the only reported B_{12}-dependent enzyme catalyses the final step in methionine biosynthesis—the methylation of homocysteine to give methionine. These bacteria do not depend on exogenous B_{12} for growth, however, for they have an alternative B_{12}-independent homocysteine transmethylase (non-B_{12} enzyme) which can catalyse the same reaction. The non-B_{12} transmethylase is much less efficient than the corresponding B_{12}-dependent enzyme: in bacteria grown in the absence of vitamin B_{12}, the non-B_{12}-dependent enzyme comprises 3–5% of the total soluble protein[2]. If this enzyme is blocked by mutation, the mutant bacteria require either methionine itself or exogenous B_{12}, which allows them to utilise the B_{12}-dependent enzyme for methionine bio-synthesis[3]. We have sought other B_{12}-dependent enzymes in *S. typhimurium* and found evidence for an ethanolamine deaminase. We did not find other examples of parallel non-B_{12}-dependent and B_{12}-dependent enzymes in *S. typhimurium*. *E. coli* also seems to have an ethanolamine deaminase.

We first searched for B_{12}-dependent catabolic enzymes, since other bacteria use such enzymes in major catabolic pathways for arginine, lysine, ornithine, propanediol, glycerol, glutamate and nicotinate[4]. If *S. typhimurium* or *E. coli* have similar B_{12}-dependent catabolic pathways, they would have gone unnoticed in surveys of utilisable carbon and nitrogen sources performed in the absence of exogenous B_{12}. Petri dishes of carbon-deficient or nitrogen-deficient medium were seeded with about 2×10^8 cells of wild type *S. typhimurium* LT 2 and a few µg of each test compound was added to the surface of the plate and incubated at 37 °C (ref. 5). Each substance was tested in the presence or absence of vitamin B_{12} (cyanocobalamin, 0.4 µg ml^{-1}). Growth was observed daily for a week. Ethanolamine was found to serve as a source of nitrogen in the presence of vitamin B_{12}.

With B_{12}, ethanolamine supported as much growth as did ammonium. The final growth yields were the same, regardless of whether ethanolamine or NH_4Cl was used as the nitrogen source in a minimal medium containing vitamin B_{12}, glucose and
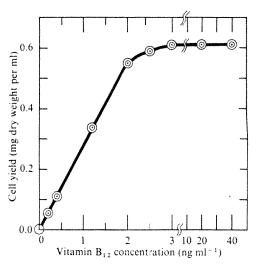
Fig. 1 Growth of *Salmonella* on ethanolamine and vitamin B_{12}. The growth medium was T2 salts mixture[8] supplemented with 0.5% glycerol, 7.4 mM ethanolamine hydrochloride, and the indicated quantities of vitamin B_{12}. Cultures were shaken for at 37 °C. After 34 h of incubation, cell yields were determined by measuring the 650 nm absorbance of cell suspensions.

salts. With ethanolamine, the doubling time was about 10 min, with NH_4Cl, about 50 min.

Vitamin B_{12} was essential for the utilisation of ethanolamine At low B_{12} concentrations, the growth of *S. typhimurium* wa proportional to the concentration of vitamin (Fig. 1). Colbaltou chloride did not satisfy the vitamin B_{12} requirement, indicatin that the synthesis of B_{12} compounds in *Salmonella* is not limite by the availability of cobalt.

Ethanolamine may also serve as a poor carbon source wit B_{12}. After 36 h of incubation on carbon-deficient solid medium there was slight growth around filter paper disks saturated wit a solution of ethanolamine.

Many members of the Enterobacteriaceae may be able t utilise ethanolamine as a nitrogen source in the presence o exogenous vitamin B_{12}. *E. coli* (K12) and *Enterobacter aerogene* (ATCC 1033) showed the same response as *S. typhimurium* t vitamin B_{12} and ethanolamine. Turner *et al.* have reported[6] tha *Erwinia* spp. utilise ethanolamine as a nitrogen source, but ther is no specific evidence for a B_{12}-dependent pathway.

We next used penicillin selection procedures to determin whether homocysteine transmethylase is the only system i which *S. typhimurium* has alternative B_{12}-dependent and non B_{12}-dependent enzymes. If other systems are catalysed b alternative B_{12}-dependent and non-B_{12}-dependent enzymes, i should be possible to inactivate the latter enzymes by mutation mutants lacking the non-B_{12}-dependent enzyme should requir exogenous B_{12} for the corresponding B_{12}-dependent enzyme Mutants were induced by saturating a fully grown culture o *S. typhimurium* LT 2 with diethylsulphate and incubating it fo 10 min. A sample (0.1 ml; 2×10^8 cells) of this culture was the transferred to 5 ml of nutrient broth and grown overnight. The 0.1-ml portions of the culture were collected on Millipore filter and subjected to selection[7]. After treatment with penicillin, th cells were filtered, washed and grown in a minimal glucos medium supplemented with vitamin B_{12}. Clones derived from these cultures were tested for their ability to grow on minima glucose medium with and without methionine or vitamin B_{12} supplements. All incubations were at 37 °C. From 217 survivor of the selection procedure, we obtained 89 B_{12}-requirin mutants, all of which grew in the presence of either vitamin B_{12} or methionine. They were presumably all *metE* mutants whic lack the non-B_{12} enzyme in the methionine pathway.

We then modified the penicillin selection procedure to exclud methionine mutants. Methionine (0.2 mM) was added to th penicillin solution. Any methionine auxotrophs would then b killed by the penicillin. Any B_{12}-requiring mutants which do no respond to methionine, would presumably have lost the non-B_1

)art of some metabolic system which normally has alternative B_{12}-dependent and non-B_{12}-dependent enzymes We obtained 10 mutants of this type, even after two sequential selection operations on four mutagen-treated cultures This technique should have yielded mutants occurring at frequencies as low as 10^{-8} per mutated cell

The failure to obtain mutants in the second series of penicillin selection experiments suggests that only the methionine biosynthetic system has alternative B_{12}-dependent and non-B_{12}-dependent enzymes Other explanations for our failure to isolate mutants could be (1) the penicillin selection procedure was not as efficient as expected, (2) the traces of B_{12} found in *S typhimurium* were sufficient for growth, thus preventing the isolation of B_{12} auxotrophs, or (3) the systems using alternative B_{12}-dependent and non-B_{12}-dependent enzymes are not needed for growth under laboratory conditions The first explanation is unlikely, however, since so many B_{12}-requiring methionine mutants were isolated in the first selection experiments The second explanation is unlikely unless the hypothetical B_{12} enzyme has a much higher affinity for B_{12} and is required in much smaller amounts than the methionine biosynthetic enzyme

Our nutrition experiments suggest that *S typhimurium* and *E coli* have a B_{12}-dependent ethanolamine deaminase (ethanolamine–ammonia–lyase, EC 4 1 3), analogous to the enzyme in clostridia[8] It seems pertinent that these organisms have two B_{12}-dependent metabolic pathways and yet they cannot synthesise significant amounts of the B_{12}-compounds Under the usual laboratory conditions, neither pathway is essential for growth methionine can be made by the B_{12}-independent pathway and ethanolamine is not usually needed as a nitrogen source Nevertheless, the fact that the bacteria have both pathways suggests that they are at times advantageous and that the bacteria often have exogenous supplies of B_{12} compounds This is not surprising, since faeces and sewage are rich sources of vitamin B_{12} and related compounds

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Drugs blocking the muscle-damaging effects of 5-HT and noradrenaline in aorta-ligatured rats

USING an animal system proposed as a model for the hypothesis that Duchenne muscular dystrophy might be caused by an impaired blood supply within muscle[1-3], we have found that certain drugs prevent the muscle damage typical of this disease The model involves combining ligature of the aorta with a minimal dose of serotonin (5-hydroxytryptamine, 5-HT) or noradrenaline to produce myopathy of the lower extremities, which is evident in histochemical changes[3] and increased amounts of some muscle enzymes in the plasma[4]

Imipramine (IP), phenoxybenzamine (PBZ) or chlorproma-

zine (CP), dissolved in normal saline, were injected intraperitoneally into 793 male Osborne–Mendel rats (150–175 g) which had been double-ligatured below the renal arteries[3,4] Beginning on the fifth day after ligature, the animals were pretreated with daily injections of one drug for 3 d On the eighth day after ligature, 8 h after the last injection, half the animals were given a dose of 5-HT (10 mg kg^{-1}) and half were given noradrenaline (3 25 mg kg^{-1}) Twelve hours after the 5-HT or 5 h after the noradrenaline they were bled terminally into preheparinised syringes by cardiac puncture under light pentobarbital anaesthesia The blood was cooled and cold-centrifuged at 17,750g for 2 min, and the plasma was pipetted off for enzyme analysis

For glutamic-oxaloacetic transaminase (GOT) we used the Reitman–Frankel method (U ml^{-1}), for lactic dehydrogenase (LDH), the Babson–Phillips method (IU), for creatine phosphokinase (CPK), the Rosalki method (μU ml^{-1}), and for alkaline phosphatase, the Babson–Phillips method (U ml^{-1})[4] The drugs were given to groups of animals in scaled doses IP (297 animals) 1 25, 2 5, 5, 10, 20 mg kg^{-1}, CP (301 animals) 2 5, 5, 10 mg kg^{-1}, PBZ (195 animals) 2 5, 5, 10, 20 mg kg^{-1}

Immediately after ligature the first four plasma enzymes increased prominently but all returned to normal by the fifth day[4] (40 animals) 5-HT or noradrenaline in the doses used, when not preceded by ligature of the aorta, caused no significant increase in enzyme activity[4] Peak increases occurred in ligatured animals 12 h after 5-HT (ref 4) and 5 h after noradrenaline (determined in this study using 52 animals analysed 1, 2, 3, 4, 5, 8, 12 and 16 h after injection) In neither the test-control group nor any of the ligatured animals given IP, PBZ or CP before 5-HT or noradrenaline was there a significant increase in alkaline phosphatase, suggesting that liver damage was not the cause of plasma GOT, GPT, CPK and LDH increases For the purpose of this study we therefore term them muscle enzymes Injections of ligatured animals for 3 d with the largest doses of IP, PBZ or CP alone did not alter plasma enzyme values Animals receiving IP and PBZ (housed 3–5 per 27×44 cm plastic box) were not noticeably more active or less active than controls, but those given CP were slightly sluggish

The increase in all four plasma muscle enzymes provoked by a single dose of 5-HT was prevented, in a consistent relation to dose, by prior IP, PBZ or CP (Figs 1–3), the most complete effect was with IP (10 and 20 mg kg^{-1}), subsequent doses of 5-HT having almost no effect on enzyme activity (Fig 1) The increase in muscle enzymes was prevented in a similar manner when PBZ and CP were given before noradrenaline (Figs 2 and 3) IP before noradrenaline, however, had two opposite effects, depending on the dose small doses (1 25 and 2 5 mg kg^{-1}) significantly prevented the induced increase of all the enzymes, while the largest dose (20 mg kg^{-1}) markedly enhanced the increase of CPK and LDH (and had no net effect on GOT and GPT) Thus, the largest dose of IP had opposite effects on the presumably indirect muscle-damaging actions of 5-HT and noradrenaline (Fig 1)

The muscle damage caused by low doses of 5-HT or noradrenaline given to aorta-ligatured rats is probably the result of ischaemia[1 3] The protective effect of pretreatment observed with PZB or CP before noradrenaline or 5-HT may be related to the known ability of both to block the action of α-adrenergic agents as well as responses to 5-HT[5-7] The enhancing effect of high doses of IP on muscle damage induced by noradrenaline may be related to the known ability of IP to reduce uptake of noradrenaline into nerve terminals[5,8] Since IP also reduces such uptake of 5-HT[9,10], the reason for its action in blocking the 5-HT effect in our animals at all doses and the noradrenaline effect in low doses must be explained otherwise, perhaps related to the following IP often causes orthostatic hypotension and it can antagonise or reverse the local vasoconstrictor action of noradrenaline[6], presumably by α blocking (although desmethyl-IP can block release of noradrenaline from nerve endings[5]), and it can block the spasmogenic effects of 5-HT on the smooth muscle of isolated guinea pig ileum[6] It is possible that, at certain IP doses, analogous mechanisms predominate in the
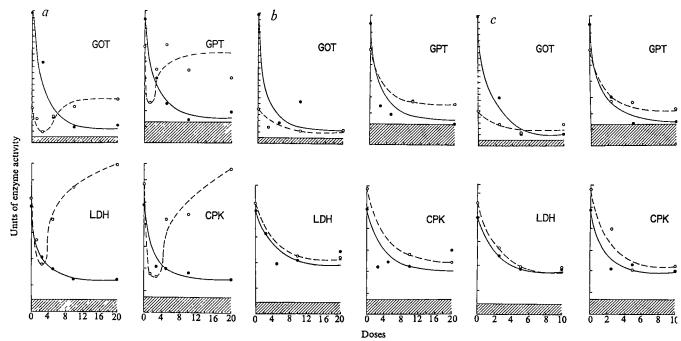
Fig. 1 Mean values (40–75 animals for each point) of plasma enzyme levels of GOT, GPT, LDH and CPK in aorta-ligatured rats given a single dose of 5-HT (●) or noradrenaline (○), pretreated by *a*, imipramine, *b*, phenoxybenzamine, or *c*, chlorpromazine Doses of pretreatment drugs on abscissa are given in mg kg^{-1} Divisions on the ordinate are GOT 100, GPT 20, LDH 100 and CPK 100 U of enzyme Zero point on the abscissa indicates enzyme level in control animals ligatured and given 5-HT or noradrenaline but no pretreatment drug, cross-hatched region indicates maximal upper range (on ordinate) of enzyme level in normal ligature-only rats, which is the same as in normal rats

smooth muscle of the abnormal (underperfused) intramuscular arterial vessels of our model

We conclude that this is a useful animal test system for evaluating interaction of drugs which affect muscle, presumably by acting on the intramuscular arterial system If the muscle-damaging mechanism in our model is, in principle, analogous to that in Duchenne muscular dystrophy, these experiments profile screening data of possible clinical relevance Because local metabolic factors are thought to be producing maximal vascular deconstriction in ischaemic regions of normal muscle[7], blockers of amine-induced vasoconstriction in normal persons may only be able to open collateral feeding vessels In Duchenne dystrophy muscle, however, we propose that there could be an inherited defect making small arterial vessels hyposensitive to deconstrictive local metabolic factors and/or overly sensitive to other constrictive ones such as vasoactive amines, defects for which amine-blockers might be at least partially corrective

The pertinence of our model, involving aorta ligature and vasoactive amine in low dosage, to Duchenne dystrophy has recently been questioned and an alternative one proposed— IP plus 5-HT at very high doses (10 times that used in our experiments) in non-ligatured rats[11] Pretreatment with IP had only a worsening effect (histologically) on muscle damage produced by the very high doses of 5-HT[11] Nevertheless, in Duchenne dystrophy patients we favour the mechanism/model of a small amount of vasoactive substance interacting with an underlying arterial vessel abnormality because they have no systemic evidence of large circulating amounts of vasoactive amine

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Fluorescence techniques for following interactions of microtubule subunits and membranes

There is increasing evidence that several cell functions are controlled by the state of polymerisation of microtubules (MTs) and by the interaction of MTs with membranes For example, previous studies in our laboratory have shown that colchicine-sensitive structures, presumably MTs, determine the topographical organisation of cell membrane components[1-3] Conditions for the polymerisation of MTs *in vitro* have been described[4] The MT subunit has been identified by centrifugation studies as a 6S dimer of approximately 110,000 daltons[5] An additional 36S component is required for MT assembly[6] The established procedures used, however, to record MT polymerisation (light scattering and viscosity measurements supplemented by negative staining and electron microscopy) do not reveal details of the interactions between subunits undergoing polymerisation and cannot be applied in the presence of elements such as membranes that contribute separately to light scatter and viscosity

We report here a new method to monitor MT polymerisation and to investigate interactions between MTs and membranes Our approach involves long range resonance energy transfer between different fluorescent moieties separately conjugated to different MT subunits or to membranes The method permits study of weak or readily dissociable interactions between membranes and MTs or between subunits, which may not

survive techniques that physically separate or dilute the complexes

Resonance energy transfer occurs when a chromophore which is excited by the absorption of energy transmits the energy to another (acceptor) chromophore some distance away This transmission requires the overlap of the emission spectrum of the first (donor) chromophore with the absorption spectrum of the acceptor, but it does not involve the actual reabsorption of light by the acceptor It is therefore referred to as non–radiative or resonance energy transfer Resonance transfer requires that the distance between the chromophores be relatively close (not exceeding 100 Å) and in fact quantitative theory allows one to estimate the distance between chromophores from the extent of energy transfer[7–10]

In our experiments fluorescein isothiocyanate (FITC) was used to label one population of MT subunits (protein dimers called tubulin and designated T) or membranes, and rhodamine isothiocyanate (RITC) was used to label a second population of tubulin These chromophores were chosen because they bind covalently and because the emission spectrum of fluorescein (donor) extensively overlaps the absorption spectrum of rhodamine (acceptor) In the non-polymerised state a mixture of fluorescein–labelled (F-T) and rhodamine-labelled (R-T) tubulins does not show resonance transfer With polymerisation (or aggregation) of MT-subunits, however, the chromophores are brought sufficiently close together so that energy transfer occurs The degree of transfer is thus dependent on the number of units polymerised Figure 1 shows a time course of the polymerisation of MTs followed by light scattering and resonance energy transfer The progressive increase in scatter was the same whether or not dye was conjugated with the tubulin, indicating that these low concentrations of bound chromophores do not interfere with MT assembly At the same time resonance transfer of energy from fluorescein to rhodamine was measured by exciting the sample at 480 nm, and recording the emission spectrum between 500 and 620 nm (to include
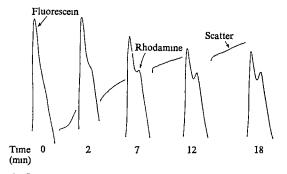
Fig 1 Time course of light scattering and fluorescence emission spectra during MT polymerisation Tubulin (T) was purified from rabbit brain by successive cycles of polymerisation and depolymerisation as described by Shalanski[11] Protein–dye conjugates were formed by incubation of purified protein (4 mg ml^{-1}) with fluorescein isothiocyanate (FITC) or rhodamine isothiocyanate (RITC) for 30 min at 4 °C Incubation was stopped by separating the conjugates from the free dye by column chromatography (Sephadex G–25) Dye to protein molar ratios (D/P) were determined by absorbance measurements using extinction coefficients of the bound dyes[12] and protein measurements by the method of Lowry[13] The absorbances of the mixtures followed spectroscopically did not exceed 0 20 absorbance units In this experiment incubations of 10 µg dye per mg protein and 5 µg mg^{-1} protein for RITC and FITC respectively gave approximate D/P values of 0 35 for R-T and 0 08 for F-T Tyndall scatter was measured at 650 nm using parallel polarised light, monitored at 90° to the incident beam (This assures a high signal to noise ratio) Viscosity changes (not shown) agreed closely with the time course of scatter measurements At intervals the latter were interrupted and emission spectra obtained by exciting the sample at 480 nm and scanning from 500 to 650 nm Corrected spectra were obtained using a Perkin–Elmer MPF4 fluorometer At zero time a single peak representing only fluorescein emission was obtained A rhodamine emission peak developed progressively as resonance energy transfer occurred between the polymerising fluorescein and rhodamine-labelled subunits See text

both fluorescein and rhodamine emissions) The sample was excited at 480 nm instead of at the excitation maximum of 496 nm to minimise the direct excitation of rhodamine Before polymerisation (Fig 1, zero time) a simple fluorescein emission spectrum was observed There was no energy transfer and thus no rhodamine emission Later (Fig 1, 2 min and later) as subunits separately labelled with F and R were brought together, resonance transfer occurred and the characteristic emission peak of rhodamine progressively increased Simultaneously, since resonance transfer and emission compete for the excited state, a decline in fluorescein emission was observed When F-T alone (no R-T present) were polymerised, no decrease in fluorescein emission occurred The depopulation of the fluorescein excited state by energy transfer to rhodamine is also reflected in a decrease in the excited state lifetime Fluorescent lifetime measurements[14] showed a decrease from 5 15 ns for MT assembled from F-T dimers alone to 3 64 ns for maximally (>70%) polymerised MTs assembled from the F-T plus R-T mixture From these data a mean distance between chromophores of 44 Å was calculated[7] The same distance was obtained by calculation from the energy transfer spectrum of maximally polymerised MT obtained in Fig 1, and is consistent with the known geometry of MTs (Fig 2)

Energy transfer did not occur when depolymerised F- and R-labelled subunits were incubated under conditions in which polymerisation did not occur (at 4 °C, or in the presence of 4 mM calcium chloride, or in the absence of GTP) But we observed interactions between subunits in the presence of colchicine, a plant alkaloid that binds with tubulin subunits and is thought to prevent their association into microtubules A tenfold molar excess of colchicine slowed markedly the increase in the light scattering None the less, in the presence of colchicine resonance transfer increased progressively with time and to nearly the same degree as in the absence of the alkaloid This indicates that most of the MT subunits are probably associated into small aggregates These aggregates seem to be readily dissociable the addition of unlabelled tubulin to the colchicine mixture, or simple dilution, led to a rapid fall in energy transfer In contrast, neither unlabelled tubulin nor dilution altered the energy transfer of polymerised MT Thus, the effect of colchicine on the interaction of subunits was different from the effects of cold or calcium which prevent polymerisation and the development of energy transfer and light scattering

Preliminary experiments have also demonstrated interactions of MT and/or tubulin with isolated cytoplasmic membranes Cytoplasmic membranes were isolated from rabbit polymorphonuclear leukocytes as previously described[8] and incubated with FITC at approximately 25 µg FITC per 100 µg membrane protein for 10 min at 4 °C The labelled membranes were then washed free of unreacted FITC by recentrifugation through a discontinuous sucrose gradient R-T was prepared as before and mixed with the labelled membrane At 4 °C virtually no energy transfer took place between F-labelled membranes and R-labelled tubulin At 37 °C polymerisation of R-T occurred, and resonance energy transfer between F-membranes and R-tubulin developed in parallel with the increase in light scatter This transfer was not diminished by the addition of unlabelled tubulin subsequent to polymerisation Similar experiments using rhodamine-labelled bovine serum albumin and fluorescein-labelled membranes did not reveal energy transfer at any temperature These experiments suggest a specific association of polymerised MT with membrane Energy transfer between R-T and F-membranes increased progressively with time in the presence of colchicine, just as it did between R-T and F-T with colchicine Further, addition of unlabelled tubulin to the membrane–tubulin complex formed in the presence of colchicine (10^{-4} M) rapidly reduced the resonance transfer Thus, in the presence of colchicine the association of tubulin with membranes, like the association of tubulin with tubulin seems readily reversible, and is distinct from the association formed in the absence of the alkaloid The high

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Fig. 2 Schematic diagram of microtubule and disposition of constituent fluorescent labelled subunits The diagrammatic tubule was generated using the known geometry of MTs[15] and distributing tubulin dimers labelled with fluorescein (F), rhodamine (R) and unlabelled dimers (N) in the tubule according to a list of random numbers The proportions of F, R and N were calculated from the D/P ratios of F-T (0 15) and R-T (0 46) measured in a maximally (> 70%) polymerised suspension of MT, assuming that the association of dye molecules with tubulin follows a Poisson distribution[16] In this mixture, the distance between F and R calculated from resonance transfer as described in the text is approximately 40 Å This is consistent with the dimensions of the MT as shown, which is made up of protein dimers with a cross-sectional diameter of roughly 40 Å This correspondence suggests that resonance transfer occurs only between immediately adjacent subunits

efficiency of energy transfer observed (> 50%) indicates considerable interaction of the tubulin and membrane, and suggests that some labelled constituent of the membrane preparation could serve as a nucleation centre for MT polymerisation

In summary the use of long range non-radiative energy transfer provides a new approach to the study of MY polymerisation This method gives information on the geometry and interactions of subunits and of interactions between MTs and other cell constituents It has been applied to demonstrate loosely associated tubulin aggregates formed in the presence of colchicine, and binding of microtubules to isolated membranes, neither of which has been observed with conventional techniques These experiments are now being extended to explore the specificity and number of membrane binding sites for tubulin under different physiological conditions. The approach should prove useful in the study of other polymerisations (for example, microfilaments), particularly of their interactions in mixed systems containing membranes

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Lateral compressibility and penetration into phospholipid monolayers and bilayer membranes

THE phospholipid bilayer[1] in certain biological membranes 1 maintained so that the range of temperature over which the gel to-liquid crystal transition occurs includes the environmenta temperature[2] As a result, clusters of phospholipid molecules u crystalline and liquid-crystalline states[1] coexist in the membran and the isothermal lateral compressibility of the membran lipids is enhanced[3] Any increase in compressibility shoul facilitate insertion of foreign molecules into the bilayer thereb affecting transport across the membrane Indeed, there 1 evidence that transport of ions[4,5] and sugars[3], and penetratio of an enzyme[6] is increased when the chain-melting transition c the lipids occurs The lateral compressibility of phospholipi bilayers has not been measured, however, and there is no direc evidence for an increase in compressibility at the point wher the gel-to-liquid crystal phase transition occurs In order t measure lateral compressibility, compression solely in the plan of the bilayer is required Such directed compression of bilayer will be difficult but it can be readily achieved with monolayers a the air–water interface Since the molecular packing in suc monolayers of phospholipids is equivalent to that in bilayer dispersed in excess water[1,7], this model system is particularl convenient for exploring the role of lateral compressibility Here we show (1) how the compressibility of lecithin mono layers varies with packing density and changes at the chain melting transition, and (2) how penetration of a hydrophobi protein is dependent on the compressibility

The procedures used to obtain the surface pressure (π) molecular area (A) isotherms for chromatographically pur 1,2-dipalmitoyl phosphatidylcholine (lecithin) have bee described before[7,8] The preparation and adsorption characteri tics of 1-[14]C-acetyl-β-casein A have also been published[9,10] th protein is very hydrophobic and its behaviour at the air–wate interface is like that of proteins which are associated with lipid *in vivo* and quite different from that of highly water-solubl globular proteins The penetration experiments were performe at constant surface area by maintaining the lecithin monolaye at an initial A with a barrier and injecting the protein into th substrate The initial substrate concentration of protein wa 1 × 10⁻⁴ g per 100 ml and the changes in π and surface concen tration of protein (Γ) were monitored during adsorption with Wilhelmy plate and gas-flow counter[11], respectively

Figure 1a shows the π–A curves for dipalmitoyl lecithi spread at 20 °C and 30 °C on to a phosphate buffer (I = 0 1 pH 7), the isotherms are similar to those for a 0 1 M NaC substrate[7] The inflection at π = 5 6 mN m⁻¹ and A = 77 Å² pe molecule (this point is difficult to reproduce and is sensitive t the rate of compression) is a result of the onset of two-dimen sional crystallisation[1,7] and over the region where the π–A curv is more-or-less horizontal the monolayer consists of a mixture o crystalline and liquid-crystalline domains Because the transi tion is equivalent to that occurring in bilayers, the critica temperature[1,7] for the monolayer transition is the same as th bilayer transition temperature (41 °C) The monolayer phas transition is probably best described as "diffuse" first order[12,1] because the compressibility (C = −1/A(δA/δπ)_T) plotte as a function of A (Fig 1b) does not become infinite at the onse of condensation[17] The form of the plot of C against A is th

ame as that generally found for monolayers which undergo the phase transition[17] The dependence of C on the phase of the ipalmitoyl lecithin monolayer is similar to that generally found[16] for lipids thus C increases by a factor of about 5–0 15 1 mN^{-1} on moving from the liquid-expanded (for a definition of terms see ref 18) to the transition (intermediate[18]) region In the rystalline (condensed) region $C \sim 0\,004$ m mN^{-1}
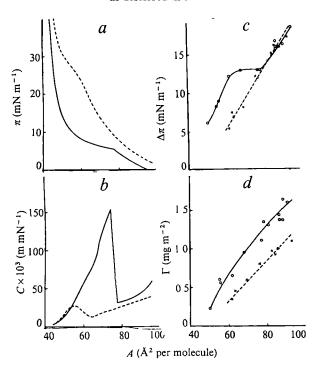
The magnitude of C is directly related to fluctuations in the total number of particles in the system[19] The high compressibility at the phase transition results from large density fluctuations in this region Because of such fluctuations in surface density π will also fluctuate and Blank[20] has shown that the instantaneous surface pressure is related to the mean value $\langle \pi \rangle$ by equation (1)

$$\pi \simeq \langle \pi \rangle [1 \pm (kTC/A)^{\frac{1}{2}}] \qquad (1)$$

As a consequence of the enhanced lateral compressibility in the transition region, when $\langle \pi \rangle \sim 20$ mN m^{-1} (which is characteristic of phospholipid bilayers[1]) π can fluctuate locally between 3 and 37 mN m^{-1} Fluctuations which give rise to a low π are equivalent to hole formation in the monolayer, such holes lead to enhanced permeation of small molecules[20,21]

High values of C also enhance penetration of large molecules such as proteins which compress the lipid to clear a space (X) for themselves In this case, penetration is opposed by an activation energy barrier $E = \langle \pi \rangle X$ Penetration continues with increasing $\langle \pi \rangle$ (Fig 1c) until E becomes too large, generally this point is reached and penetration ceases when $\langle \pi \rangle \sim 30$ nN m^{-1} (refs 22, 23) The area which can be cleared by compression of the lipid for a given increase in $\langle \pi \rangle$ is obviously proportional to C As a result it is to be expected that protein

penetration is enhanced when C is high That this is indeed the case can be seen from Fig 1d It is apparent that Γ for β-casein is higher at 20 °C than at 30 °C (Γ for β-casein at the air–water interface is independent of temperature over this range) because the phase transition is prominent at 20 °C and C is much greater (Fig 1b) We could not detect any differences in the rate of increase of Γ at the two temperatures

The increase in π on penetration of protein (Fig 1c) arises from the increase in packing density in the interface The molecular packing in the mixed film can be described by a model[9,24] in which it is assumed that the protein penetrates and condenses the lecithin molecules to give a mosaic structure The detailed packing of the dipalmitoyl lecithin molecules in the mixed film cannot be determined from the present data The initial physical state of the lecithin monolayer is important through its effect on C, for example the initially condensed monolayers formed by higher lecithin homologues[7] give rise to a small degree of penetration with β-casein[9,24] Consistent with this, the dashed lines in Fig 1c and d extrapolate to zero $\Delta\pi$ and Γ when $A \sim 47$ Å^2 per molecule, at this point the phospholipid molecules are closely packed and there is no free space in the film into which protein molecules can penetrate The presence of cholesterol which leads to the formation of condensed monolayers with lecithin[1] also reduces C and should therefore inhibit protein penetration (compare ref 25)

We can conclude that the lateral compressibility of phospholipid monolayers and bilayers is an important parameter in determining their permeability If in vivo a biological membrane is maintained so that the phospholipid bilayer contains bo h crystalline and liquid-crystalline regions in equilibrium then the resultant enhancement of lateral compressibility facilitates insertion of hydrophobic proteins As a result the concentration of such proteins inside the membrane can be increased and if they are functional in transport, then the membrane permeability rises (compare ref 3) Besides processes such as membrane transport and assembly (biogenesis), the high lateral compressibility arising when a biological membrane is maintained in the range of the gel-to-liquid crystal transition may be of fundamental importance for membrane processes in general A phospholipid monolayer at the air–water interface is a particularly convenient model for quantitative studies of the role of lateral compressibility The elucidation of the exact role of high lipid compressibility in membrane biology warrants a systematic investigation

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Fig 1 *a*, Surface pressure (π)–molecular area (A) isotherms for dipalmitoyl lecithin at the air–phosphate buffer (pH 7, $I = 0\,1$) interface at 20 °C (——) and 30 °C (– – –) *b*, Variation of isothermal lateral compressibility of dipalmitoyl lecithin monolayers with molecular area at 20 °C (——) and 30 °C (– – –) *c*, The dependence of the change in surface pressure ($\Delta\pi$), after penetration of 1-^{14}C-acetyl-β-casein, on the initial molecular area of dipalmitoyl lecithin monolayers at 20 °C (O) and at 30 °C (×) The substrate was phosphate buffer and the initial substrate concentration of β-casein was 1×10^{-4} g per 100 ml *d*, The dependence of the final surface concentration (Γ) of 1-^{14}C-acetyl-β-casein on the initial molecular area of dipalmitoyl lecithin monolayers at 20 °C (O) and at 30 °C (×) The other conditions were as described in *c*

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Amino acid transport defect in glutathione-deficient sheep erythrocytes

THE tripeptide, reduced glutathione (GSH), is present in high concentration in mammalian red blood cells, where its major role is thought to be the protection of the cell against oxidative damage Congenital red cell GSH deficiency has been reported in man, and attributed to a decreased activity of either of the two enzymes (γ glutamyl cysteine synthetase (GCS) or GSH synthetase (GSHS)) involved in GSH biosynthesis[1] Such GSH-deficient red cells have a markedly diminished lifespan, and an increased susceptibility to the haemolytic action of oxidant drugs[1]

Sheep with low concentrations of red cell GSH were first found by Smith and Osburn[2] and subsequent investigations have revealed at least two distinct types of congenital sheep red cell GSH deficiency[3-5] The first is associated with a diminished activity of the first enzyme of GSH biosynthesis (GCS) and is found in Tasmanian Merino sheep ("Merino-type")[6] The second, found in Finnish Landrace sheep ("Finn-type"), is characterised by normal activities of both GCS and GSHS[6], and results in a shortened red cell lifespan[7], and an increased susceptibility to kale poisoning[8] Significantly, in the latter, but not in the former type of deficiency, the red cells contain high concentrations of certain amino acids, particularly lysine and ornithine[5] It is therefore possible that the low concentrations of GSH in these animals may result from a reduced amino acid permeability limiting the availability of GSH precursors, or from a direct effect of the accumulated amino acids on the enzymes of GSH biosynthesis

To test the first possibility we have measured the permeability of normal (high GSH) and GSH-deficient (low GSH) Finn red cells to the GSH-precursor amino acids (cysteine, glutamate, glycine) and compared the results obtained with those for high and low GSH Merino cells The data are presented in Table 1 together with values for glutamine, α-amino-n-butyric acid (αAB), cystine and lysine uptake by high and low GSH Finn red cells The uptake of cysteine was rapid in Finn red cells, and sixfold greater in high GSH cells than in low GSH ones In contrast, the uptake of cysteine by high and low GSH Merino red cells was the same, and not significantly different from that found in high GSH Finn cells As with cysteine, glycine uptake was significantly lower in low GSH Finn red cells, although both the absolute fluxes and the difference between the GSH types were smaller There was no significant difference in glycine permeability between high and low GSH Merino red cells Attempts to demonstrate glutamate uptake by sheep red cells gave very low values, which were not linear with time The impermeability of sheep red cells to glutamate is consistent with observations on human red cells[9] Glutamine may act as a GSH precursor[10] and measurements of glutamine uptake showed fluxes of the same order as for glycine, but with no significant difference between Finn GSH types

In certain reactions (including that catalysed by GCS), αAB can substitute for cysteine[11,12] and may therefore provide a convenient alternative to cysteine for uptake studies. The

Table 1 GSH levels and amino acid uptake rates in red cells from high and low GSH Finnish Landrace and Tasmanian Merino Sheep

	High GSH Finn	Low GSH Finn	Significance level for difference (P)
No of animals	5	5	
Red cell GSH (mmol per l packed cells)	2 97± 0 24	1 25±0 08	
Amino acid uptake (µmol per l packed cells per h)			
Cysteine	236±21	38 7±4 0	<0 001
Glycine	12 7± 1 8	7 3±0 4	<0 002
Glutamic acid	<1	<1	NS
Glutamine	11 9± 1 3	13 5±3 1	NS
α-amino-n-butyric acid	656± 69	56 4±8 6	<0 001
Cystine	8 6± 0 4	9 9±0 5	<0 1
Lysine	19 4± 3 0	6 4±0 8	<0 01

	High GSH Merino	Low GSH Merino	
No of animals	6	6	
Red cell GSH (mmol per l packed cells)	3 19± 0 04	0 87±0 04	
Amino acid uptake (µmol per l packed cells per h)			
Cysteine	298±12	242±2	NS
Glycine	10 4± 0 8	11 7±0 6	NS

Data presented as mean ±s e m
NS, not significant
Washed sheep red cells were incubated at 10% haematocrit in a medium containing (mM) NaCl 135, KCl 5, Tris-HCl(pH 7 1 at 37 °C) 15, $MgCl_2$ 3 1, EDTA 0 1, amino acid 0 2 (containing ^{14}C-amino acid at 0 2 µCi ml^{-1}) Since cysteine oxidises rapidly in solution at neutral pH, cysteine uptake was measured in the presence of 10 mM dithiothreitol Control experiments showed no effect of 10 mM dithiothreitol on αAB influx or efflux At fixed time interval (10–30 min for Gly, αAB, Lys, Cys/2, 30–90 min for Cys, Gln Glu), aliquots (1 ml) were taken and the cells washed four times in ten volumes of a medium containing (mM) $MgCl_2$ 106, Tris-HCl (pH 7 4 at 4 °C) 10, by centrifugation (10 s, 10,000g) in an Eppendorf 3200 microcentrifuge Finally, the packed cells were lysed in 0 5% Triton X-100 in water (0 5 ml), 33% trichloracetic acid added (0 5 ml) and the precipitate removed by centrifugation An aliquot of supernatant (0 9 ml) was placed into Bray's solution[14] (10 ml and counted in a β scintillation spectrometer with quench correction

uptake of this amino acid by Finn red cells was therefore measured, and found to be rapid, with the flux in high GSH cells some tenfold greater than that encountered in low GSH cells In contrast to the situation with cysteine and αAB, cystine (the oxidised form of cysteine) showed a slow rate of uptake, with no significant difference between high and low GSH Finn cells The uptake of lysine, which is found at concentrations of up to 20 mmol per litre packed cells in low GSH Finn red cells was measured in high and low GSH Finn cells High GSH cells showed a threefold greater lysine permeability than low GSH cells

The above results show that differences in amino acid transport, particularly for cysteine, αAB and lysine exist between high and low GSH Finn red cells In contrast to glutamate and glycine, the concentration of cysteine in sheep red cells is very low (13 µmol per litre packed cells)[13] Thus a diminished cysteine availability may be responsible for the low concentrations of GSH in low GSH Finns

The reduced amino acid transport in low GSH Finn red cells is not a direct consequence of a low intracellular GSH concentration, since low GSH Merino red cells show normal amino acid uptake values Further experiments were designed to determine whether the diminished amino acid uptake by low GSH Finn red cells resulted from a membrane transport defect or whether it was a function of the high lysine and ornithine content of these cells Uptake of αAB (0 2 mM) by high GSH Finn red cells was unaffected by the presence of extracellular lysine or ornithine (10 mM) Similarly, αAB efflux from the same cells (preloaded by incubation in the

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esence of 1 mM αAB for 2 h at 37 °C) was unaffected by
tracellular lysine or ornithine at a concentration of 5 mM
contrast, 10 mM cysteine inhibited αAB uptake by 50%
d stimulated αAB efflux by 30% under equivalent conditions
inally, high GSH Finn red cells were preloaded with lysine
y incubation in the presence of 10 mM lysine for 20 h at
) °C Uptake of αAB (0 2 mM) by these cells (intracellular
sine concentration 3 0 mmol per litre packed cells) was identical
that of control high GSH cells which had been incubated
r the same length of time in the absence of lysine (intra-
ellular lysine concentration 0 1 mmol per litre packed cells)

We therefore conclude that the diminished amino acid
ptake of low GSH Finn red cells represents a membrane
transport defect which is not a consequence of the low GSH
r elevated lysine and ornithine concentrations in these cells
: further seems likely that diminished availability of cysteine
responsible for the low concentrations of red cell GSH in
iese animals, and that the accumulation of lysine and orni-
iine is a further reflection of reduced amino acid transport

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New type of infectious complex of *E. coli* RNA phage

REPARATIONS of the three components of RNA phage—RNA,
oat protein and maturation protein (A protein)—have been
ised in several attempts to reconstitute the particles *in vitro*[1-7]
Particles recovered so far have been similar to authentic particles
with respect to S value, morphology and other properties We
iave now reconstituted a complex, by mixing RNA and A
protein only, which can infect *Escherichia coli* regardless of its
small S value, 28S

Preparation of phage RNA and A protein, and conditions for
reconstitution are described in the legends to Table 1 and
Fig 1 Polyacrylamide gel electrophoresis showed that A
protein was not contaminated by coat protein Infectivity of
MS2 RNA-MS2 A protein complex, measured as described in
he legend to Table 1, was detected only when RNA and A
protein were mixed, and it seemed to increase when the mixture
was dialysed The greatest efficiency of infectivity observed was
approximately 1 4 × 10⁻⁵ plaque-forming units (PFUs) per
RNA strand (after dialysis) Sucrose gradient centrifugation
of the mixture gave peaks of absorbance and infectivity corres-
ponding to those of RNA (Fig 1a and b) This suggests that the
major component of the reconstituted complex is a single
molecule of RNA, and that one or a few molecules of A protein
play some roles in infecting intact host cells

By assaying infectivity of the complex on *E coli* K12 Hfr Q13,
F⁺ A/λ, F⁻ W3110 and C15 (a derivative of A/λ which is
resistant to MS2 and GA phages), we found that the host range
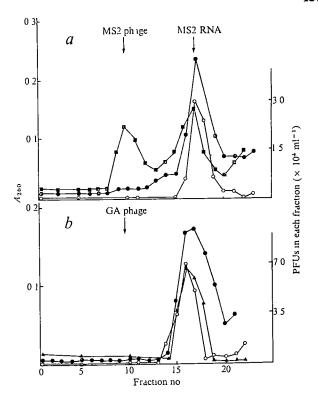
Fig 1 Formation of RNA–A protein complex P hage RNA
and phage protein were isolated from purified phage by the
SDS-phenol method[12] The isolated RNA was purified further by
sucrose gradient centrifugation and ethanol precipitation The
phage mixed protein (coat protein and A protein) was recovered
from phenol layer by the method of Hung *et al*[4], and dissolved
in 5 M guanidine-HCl, plus 0 05 M mercaptoethanol in 0 1 M
Tris-HCl buffer (pH 7 2) A protein was isolated from MS2
phage and GA phage by the method of Osborn *et al*[13] A protein
was dissolved in 5 M guanidine-HCl, plus 0 05 M mercapto-
ethanol in 0 1 M Tris-HCl buffer (pH 7 2) A 1-ml sample of
reaction mixture contained the following components, 5 μg of
MS2 (a) or GA (b) A protein, 100 μg of MS2 (a) or GA (b) RNA
(dissolved in 0 01 M Tris-HCl (pH 7 2), 0 005 M MgCl₂), 5 mmol
of guanidine-HCl, and 50 μl of mercaptoethanol in 0 1 M
Tris-HCl buffer (pH 7 2), and 200 μg of phage mixed protein if
used After dialysis as in Table 1, the reaction mixture was
centrifuged in a 5 -20% (w/v) linear sucrose density gradient at
1 °C for 4 7 h (25,000 r p m) in a Spinco SW 27 rotor Fractions
were collected from the bottom of the tube and analysed for
infectivity and absorbancy at 260 nm *a* ■, MS2 RNA+MS2
A protein + MS2 mixed protein, ●, absorbance of MS2
RNA–MS2 A protein complex, ○, infectivity of MS2 RNA–
MS2 A protein complex, *b* ●, absorbance of GA RNA–GA
A protein, ○, infectivity of GA RNA–GA A protein complex,
▲, absorbance of GA RNA

of the complex was identical to that of the phage from which
RNA and A protein were prepared

The sensitivity of the complex (MS2 RNA–MS2 A protein) to
anti-MS2 serum was tested and, unlike intact MS2 phage

Table 1 Infectivity of MS2 RNA–MS2 A protein complex

Assay mixture	PFU/RNA(μg)
RNA (before and after dialysis)	< 10²
A protein (before and after dialysis)	< 10²
RNA+A protein (before dialysis)	6 × 10⁴
RNA+A protein (after dialysis)	2 2 × 10⁵

A 1-ml sample of reaction mixture contained the following com-
ponents 5 μg of MS2 A protein, 50 μg of MS2 RNA (dissolved in
0 01 M Tris-HCl (pH 7 2), 0 005 M MgCl₂), 5 mmol guanidine HCl,
and 50 μl of mercaptoethanol in 0 1 M Tris-HCl buffer (pH 7 2)
If necessary the mixture was dialysed against Tris-acetate buffer
(0 1 M Tris-HCl (pH 7 2), 0 05 M KCl and 0 02 magnesium acetate)
in an ice bath for 18 h PFUs in the reaction mixture were assayed
on K12 F⁺ A/λ

Table 2 Sensitivity of MS2 RNA–MS2 A protein complex to anti-MS2 serum

	Control (PFU ml⁻¹)	+Anti-MS2 serum (PFU ml⁻¹)
Complex	3×10^5	5×10^5
MS2 phage	$1\,1 \times 10^7$	$4\,1 \times 10^4$

A 0 9-ml sample of a solution of MS2 RNA–MS2 A protein complex or MS2 phage suspension was mixed with 0 1 ml of anti-MS2 serum (K value = 5) and incubated at 37 °C for 10 min Remaining PFUs were assayed on K12 F⁺ A/λ In control, the complex or the phage was similarly treated in the absence of the serum

particles, the complex was resistant to the serum (Table 2)

The complex was much more sensitive to RNase, trypsin and Pronase than were intact phage particles (Table 3) The extreme sensitivity to RNase suggests that a naked RNA is a component of the complex

Analysis of the physical nature of the complex indicated that it was rather unstable Infectivity decreased to one-tenth after storage for 24 h at 4 °C Also heat pretreatment of the RNA destroyed infectivity Heating at 70 °C for 10 min in TM buffer (0 005 M MgCl₂ in 0 01 M Tris-HCl, pH 7 2) decreased the activity to one-eighth to one-twentieth, and at 100 °C to 10^{-4} or less

We conclude that a complex infectious to intact *E coli* cell

Table 3 RNase, trypsin and Pronase-sensitivity of MS2 RNA–MS2 A protein complex

	Survivors (PFU⁻¹ ml)	
RNase (μg ml⁻¹)	Complex	MS2 phage
10^{-1}	$<10^2$	$5\,4 \times 10^8$
10^{-2}	$<10^2$	NT
10^{-3}	$<10^2$	NT
10^{-4}	$<10^2$	NT
—	$1\,4 \times 10^4$	6×10^8
Trypsin (μg ml⁻¹)		
10	$<10^2$	6×10^8
1	$<10^2$	$5\,5 \times 10^8$
0 1	8×10^2	NT
0.01	$1\,6 \times 10^3$	NT
—	8×10^3	6×10^8
Pronase (μg ml⁻¹)		
50	$<10^2$	6×10^8
25	$<10^2$	NT
10	$<10^2$	6×10^8
1	9×10^2	NT
—	8×10^3	6×10^8

A sample of 0 1 ml of a solution of MS2 RNA–MS2 A protein complex was mixed with 0 9 ml of enzyme solution (Pronase-P, Kaken Chemical Co , Trypsin-Type 1, Sigma RNase-Type 1-A, Sigma) dissolved in TM (0 01 M Tris-HCl (pH 7 2), 0 005 M MgCl₂), and treated for 10 min at 37 °C
NT, not tested

was formed by mixing RNA and A protein of RNA phage Although the structure of the complex is unknown, our data suggest that it was composed of a single naked RNA molecule and one or a few A protein molecules Such a complex is probably a minimum infectious unit of RNA phage, and coat protein is dispensable in this respect The complex might have some significance for consideration of the origin and evolution of viruses An RNA–A protein complex has been reported in infected cells and so has penetration of the complex into host cells on infection by phage[8–11] These complexes might be identical to our infectious complex reconstituted *in vitro*, although infectivity was not reported in the other cases

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Retention of tumour-inducing capacity by adenovirus DNA after cleavage by restriction endonucleases

WE have previously reported that fragments of Simian aden virus SA7 DNA produced by physical breakage are oncogeni These fragments had an average molecular weight correspon ing to half-molecules ($11\,4 \times 10^6$) After separation of the hea and light molecular halves by density gradient centrifugatio we demonstrated that both half-molecule preparations reta the ability to initiate tumours in newborn hamsters We su gested that this may be caused by the presence of one or mo "oncogenic regions" in both molecular halves, or by rando breaks near the centre of the DNA which could result in single small oncogenic region, residing near the centre ar occurring with equal probability in either the heavy or ligl half-molecule preparation To clarify these results we ha studied the effect of several restriction endonucleases on tl oncogenic activity of SA7 DNA These enzymes produi defined fragments of the SA7 genome rather than the heter geneous populations produced by physical shear

DNA was extracted using a phenol method from virus th had been banded twice in CsCl gradients[2] Restriction end nucleases *Eco*RI and *Eco*RII were purified from *Escherich, coli* strain R204 and R15, respectively (Dr D Jackson) End nuclease *Hin*d was purified[4] from *Haemophilus influenz* (strain Rd, Dr H Smith) Endonuclease *Hga* was obtaine from *H gallinarum* (ATCC 14385) using a modification of tl method of Takanami and Kojo[5] Endonucleases *Hpa*I an *Hpa*II were purified[6] from *H parainfluenzae* (Dr J Setlow)

The number of fragments produced by the various enzym was found to vary from two to more than twenty (Table 1 *Eco*RI was found to make a single break in SA7 DNA Th result of analytical ultracentrifuge analysis (Fig 1) of DN/ hydrolysed by *Eco*RI revealed heavy and light fragment with buoyant densities corresponding to 61 1 and 53 9% guanine + cytosine (G+C) respectively, compared with intac SA7 DNA of 58% G+C Molecular weights for the heav and light fragments were calculated[7] and corresponded t 10 7 and 11 6×10⁶ dalton, respectively The alkaline sedimen tation coefficient for the mixed fragments ($S^0_{20,w} = 26\,2$) col responded to a single strand molecular weight of 5.5×10^5 These fragments are very similar to the heavy and light half molecule fragments produced by shear, except they represen homogeneous populations resulting from breakage of th DNA at a unique site
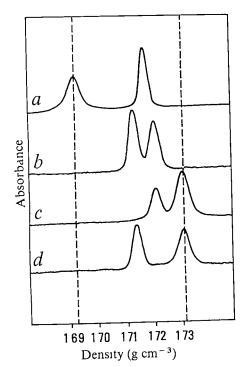
Fig 1 Model E ultracentrifuge scanner tracings of DNA preparations in CsCl-buoyant density experiments Two marker DNAs are shown *C perfringens* (ρ = 1 692) and *M lysodeikticus* (ρ = 1 731) The *Eco*RI fragments were separated on preparative NaI-EtBr gradients in a type 40 1 rotor (Beckman) by centrifugation for 100–200 h at 36,000 r p m The visible DNA bands were recovered and NaI was removed by dialysis Ethidium bromide was extracted from the dialysed DNA with isopropranol (three times) *a*, Intact SA7 DNA, ρ = 1 717, *b*, SA7 DNA cleaved by *Eco*RI, *c*, *Eco*RI heavy fragment, ρ = 1 720, *d*, *Eco*RI light fragment, ρ = 1 713

The mixture of fragments produced by each restriction enzyme was tested for oncogenicity by subcutaneous injection into newborn hamsters within 24 h of birth DNA (5 µg) was administered to each animal That cleaved by *Eco*RI or *Hpa*I retained the ability to initiate tumours (Table 1), whereas all the other preparations failed to yield tumours in the conditions tested Contamination by intact DNA could not account for tumour induction by either *Hpa*I or *Eco*RI fragments as gel electrophoresis data preclude contamination by more than 1%

Table 1 Tumour induction by SA7 DNA fragments

Enzyme	No of fragments	DNA/ animal	Tumour incidence	Length of test (d)
*Hin*d	20–25*	5 µg	0/34	365
Hga	20–25*	5 µg	0/56	85
*Hpa*I	7	5 µg	17/47	169
*Hpa*II	20–25*	5 µg	0/42	169
*Eco*RI	2	5 µg	7/35	282
*Eco*RI—Heavy		2 5 µg	4/59	120
*Eco*RI—Light		2 5 µg	0/77	120

SA7 DNA was digested with each enzyme for 1 h followed by addition of fresh enzyme and further incubation for 1–2 h In each case, the digestion products were examined using agarose or acrylamide-agarose gel electrophoresis to be certain that limit digests were obtained and that no detectable intact DNA remained (Approximately 0 01 µg SA7 DNA can be detected using EtBr staining Since 3–6 µg DNA was normally electrophoresed, intact DNA would represent at most less than 1% of the total) At the end of the digestion period, each preparation was deproteinised with phenol and dialysed against SSC (0 15 M NaCl, 0 15 M sodium citrate, pH 7 0)

* The number of fragments was estimated using electrophoretic separation 2 4% acrylamide-0 5% agarose gels More than twenty, but less than twenty-five bands could be distinguished for *Hin*d, *Hga*, and *Hpa*II

This amount of DNA per animal (0 05 µg) is insufficient to induce tumours at a frequency greater than 1% (J P B , N M , and L H , unpublished) When the heavy and light fragments produced by *Eco*RI were tested individually at a 2 5-µg dose, only the heavy fragments were oncogenic

This result demonstrates that the information required for tumour induction is probably unique and occurs only in the heavy half of the SA7 genome Thus, of the two possible explanations suggested for our previous experimental results with fragments produced by shear[1], the second alternative is more likely The existence of a single small oncogenic region of DNA near the centre of the SA7 molecule could therefore be found only in the heavy half-molecule after cleavage at a unique site by *Eco*RI, but may be found with roughly equal probability in both half-molecule preparations generated by random breaks resulting from shear (This is based on the fact that a break in the DNA molecule on the left or right side of such an oncogenic region would result in the activity being found in the light or heavy half-molecule respectively, whereas a break in the region itself may result in loss of activity) If this explanation is correct, we would expect to find the oncogenic activity near the central terminus of the *Eco*RI heavy half-molecule fragment We hope to resolve this in the near future, since we know that *Hpa*I produces six breaks in SA7 DNA, but only one of these is in the heavy *Eco*RI fragment of the molecule We are currently preparing and injecting these fragments to determine which *Hpa*I piece (or pieces) is responsible for the production of tumours by the *Hpa*I digest

Several authors have presented evidence that the transforming genes of human adenovirus type 2 may be near the left end of the Ad2 DNA molecule Gallimore *et al*[8] have analysed the DNA of rat cells transformed by Ad2 and found that some lines contain as little as 14% of the virus genome and that this region corresponds to the left end of the molecule Additionally, Graham *et al*[9] presented evidence that half-molecule fragments of human adenovirus type 5 DNA produced by shearing the DNA would transform primary rat cells in culture The transforming activity was found in the left or heavy (high G+C) half-molecule fraction They presented further evidence which suggested that the activity could be found in fragments as small as 10^6 dalton and may be localised in a region between 1 and 6% from the left end of the molecule Our results differ in the following ways First, we measured tumour induction *in vivo* rather than transformation *in vitro* Although we were able to obtain transformation *in vitro* with Ad5 DNA, we have not been able to induce tumours with either intact Ad5 DNA or *Eco*RI fragments (J P B ,* N M , and L H , unpublished) Second, we have not been able to induce tumours with SA7 fragments smaller than 5×10^6 dalton using either physically sheared material (ref 1 and J P B , N M , and L H , unpublished) or restriction enzyme fragments

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Dark-repair of ultraviolet-induced pyrimidine dimers in the DNA of wild carrot protoplasts

GREEN plants receive greater exposure to solar ultraviolet radiation and their germ cells (pollen) have a significantly greater potential for acquiring ultraviolet-induced genetic damage than do those of most animals[1] Even though cells of the higher plants can photoreactivate ultraviolet damage (refs 2–5, and my unpublished data), the absence of dark-repair capability could be a significant disadvantage since some types of excisable ultraviolet-induced DNA damage are not photo-reactivable[6] The capability for dark repair would become even more important if there were an increase in the fluence of solar ultraviolet reaching the Earth's surface[7] Attempts to demonstrate excision of pyrimidine dimers in cells of *Nicotiana*, *Haplopappus*[4], *Ginkgo*[5] and *Chlamydomonas*[8] have all given negative results These data, taken with other assays for excision repair activity (unscheduled DNA synthesis[9], repair replication[10–12]) have been interpreted as indicating a general absence of such capability in plants[12] I have now found, however, that in cultured wild carrot cells, pyrimidine dimers can be excised in the dark and that the extent of dimer excision depends on the initial number of dimers induced by the ultraviolet dose After ultraviolet fluences of about 10 J m⁻², most of the dimers are excised within 24 h, but at fluences up to 30 J m⁻², about 25% remain unexcised in DNA At fluences above 100 J m⁻², dimer excision is almost completely eliminated, perhaps due to secondary effects of these very high ultraviolet doses.

The results of dimer analyses on wild carrot DNA are presented in Fig 1 and Table 1 The yield of dimers immediately after irradiation was linear for ultraviolet fluences of 0–450 J m⁻² and was equal to 0.002% radioactivity in thymine-containing dimers per radioactivity in thymine (X []T/T) per J m⁻² dose Thus the efficiency of thymine dimer formation was 35–40% that observed in bacteria[18] and mammalian cells[19]. This difference may be attributable to the shielding effect of cytoplasmic organelles in wild carrot protoplasts

The excision data fit a curve showing 100% dimer removal at lower ultraviolet doses Essentially complete excision of up to about 0 02% X []T/T occurred within 24 h, as reported in human cells for dimer levels up to about 0 01 % X []T/T (ref 20) My data for excision in wild carrot protoplasts are minimum values compared with those from work on animal cells, since in the latter case any dead cells are eliminated from the assay because they do not adhere to the surface of the culture dish[19]

Excision was almost completely eliminated at doses above 100 J m⁻², although a few dimers were excised even at the highest doses (Table 1) The 2% excision observed after 150–450 J m⁻² of ultraviolet amounted to about 10⁵ excised dimers per cell

Alkaline sucrose gradient analysis revealed about 0 2 single-strand gaps per 10⁸ daltons of DNA appearing immediately after 35 J m⁻² 254-nm irradiation of wild carrot protoplasts (my unpublished data) This implies about 10⁴ dimer-specific endonuclease molecules per cell, if the number of gaps corresponds to the average number of repair molecules Similar data have been obtained for mammalian cells[23] A fluence of 42 J m⁻² induced approximately 8 4×10⁵ pyrimidine dimers per cell (These data are based on a 2C nuclear DNA content of 2 1×10⁻¹² g per cell[24] and on an approximation of the relative frequencies of C[]C, C[]T and T[]T dimers[8,18]) Since 58±15% of these lesions were excised in 24 h, the average rate of excision was 20,000–30,000 dimers h⁻¹, comparable with that estimated for excision repair in mammalian cells[20,23]

At 30–60 J m⁻² maximum excision (>70%) was observed after 24 or 40 h dark incubation Excision seemed to stop short of removal of all dimers, however, with no additional excision

in the longer incubation All the dimers induced by 42 J m⁻ could be photoreactivated (Table 1), so that this apparent lim for excision was not an artefact of the dimer assay procedure Clayton *et al*[25] have shown that dimers are seemingly no excised from mitochondrial DNA in three mammalian ce
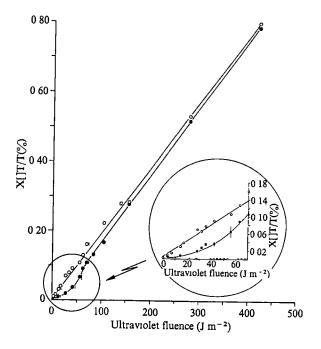
Fig. 1 Amount of thymine dimers as a function of ultraviolet fluence immediately after irradiation (○) and after ultraviolet followed by 24 h (●) or 40 h (■) dark incubation

$$X\,[]T/T = \frac{\text{radioactivity in thymine-containing dimers}}{\text{radioactivity in thymine}} \times 100$$

Standard error bars shown around the mean of three to nine separate samples Dashed line indicates background dimer level in unirradiated samples The source and culture of wild carrot cells (*Daucus carota* L) have been described[13] Cellular DNA was labelled for 20–24 h by incorporation of methyl-³H-thymidine (17 Ci mmol⁻¹, Amersham/Searle) supplied at 5 µCi ml⁻¹ in the culture medium Fluorodeoxyuridine at 10⁻⁵ M was also included to inhibit the degradation of thymidine and to enhance the specific labelling of DNA (refs 21 and 22 and my unpublished data) Cell walls were digested and protoplasts isolated and counted as before[13] The specific radioactivity (acid-insoluble) was 4±1 c p m ⁻¹ per protoplast on average Protoplasts (1 0×10⁵–1 5×10⁵ml⁻¹) were irradiated as a disperse monolayer in Petri dishes through about a 2-mm depth of protoplast isolation medium[13] with agitation at room temperature The 2-mm thickness of medium transmits about 50% of the radiation at 254 nm, therefore a correction factor[14] of 0 70 was applied to the measured ultraviolet fluences Ultraviolet was delivered by a germicidal lamp (primarily 254 nm) at a fluence rate of 0 71 J m⁻² s⁻¹ as measured with a Jagger meter[15] The use of isolated protoplasts allowed reproducible, uniform ultraviolet exposures and quantitative induction of pyrimidine dimers During and after irradiation the protoplasts were handled only under non-photoreactivating illumination Thymine-containing dimers in samples of isolated[16] DNA were analysed by two-dimensional paper chromatography[17] For the lowest ultraviolet doses, two to three times more total radioactivity (in hydrolysed DNA) was applied to each chromatogram to allow recovery of a significant level of counts in the dimer region

lines that can excise dimers induced in nuclear DNA No dimer excision was detected in *Chlamydomonas* chloroplast DNA[8] If this were the case in my wild carrot protoplasts about 20% (the approximate proportion of organellar DNA in these cells) of the dimers would have remained after maximum excision This, in fact, is the approximate limit reached after ultraviolet fluences up to about 30 J m⁻² followed by 1 d dark incubation Experiments are now in progress to evaluate the

Table 1 Representative dimer analyses in wild carrot protoplasts

Jltraviolet fluence (J m⁻²)	Dark incubation (h)	Net c p m Dimer region	Thymine region	X[]T/T (%)	Excision* (%)
14	0	277	1,560,000	0 018	
14	24	40	1,550,000	0 002	100
42	0	459	538,000	0 085	
42	24	131	338,000	0 039	56
42	†	2	180,000	0 001	(100)†
105	0	664	297,000	0 224	
105	24	580	352,000	0 165	27
280	0	1431	271,000	0 529	
280	24	724	140,000	0 516	2 5
420	0	1870	234,000	0 797	
420	24	1570	200,000	0 784	1 6

*Background (0 0034±0 0007% X[]T/T, n = 5) subtracted
†Complete photoreactivation of pyrimidine dimers by 24 h incubation under about 300 foot-candles cool-white fluorescent light (25 °C)

ossibility that pyrimidine dimers are not excised from organellar DNA in these cells

Although it is clear that pyrimidine dimers can be excised from the DNA of isolated protoplasts of cultured wild carrot cells, the distribution of this capacity among higher plants has yet to be determined Wild carrot protoplasts show a greatly reduced level of dimer excision when there are many dimers present (that is, initial levels greater than about 0 1% X[]T/T) This may explain previous failures to detect excision in tobacco (0 40% X[]T/T initial dimer level)[4], *Haplopappus* 1 12%)[4], *Ginkgo* (2 22%)[5] and *Chlamydomonas* nuclear equivalent to 0 33–0 66%)[8] and chloroplast DNAs (equivalent o 2 31%)[8] Other reports indicating an absence of excision epair activity in higher plant cells[9–12] must now be re-evaluated

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Note added in proof Soifer and Cieminis have reported dimer excision in whole grass pea, *Lathyrus sativa* L) embryos[26]

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Progressive decrease in protein synthesis accuracy induced by streptomycin in *Escherichia coli*

THE passage of genetic information from gene to polypeptide does not proceed with perfect fidelity[1–3], but it is not known what short and long-term cellular responses may be induced by decreases in the accuracy of protein synthesis Errors in gene expression may tend to increase their own rate of production[4], and thus could constitute a limiting instability of cellular life The synthesis of inaccurate RNA, RNA polymerases, tRNAs, tRNA modifying enzymes, tRNA charging enzymes and ribosomal proteins are potential pathways for the perpetuation or amplification of errors Such errors may be inducible *in vivo* by external chemical agents and may constitute an indirect pathway for mutagenesis[5–8] An enhanced rate of such errors may be the mechanism by which certain deleterious mutations exert their
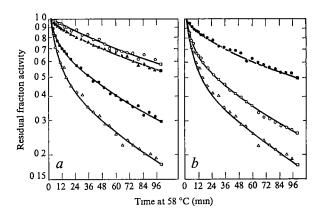
Fig 1 Streptomycin-induced, time-dependent changes in the thermal stability of β-galactosidase A culture of a streptomycin-sensitive, lac permeaseless derivative of the K12 strain W1485 (called IH79) was grown at 37 °C in enriched medium (Luria broth and phosphate-buffered minimal A, as described in Miller[10], 1 1) to the mid-log phase and maintained there during the experiment by dilution into prewarmed medium Streptomycin (2 μg ml⁻¹) was added at generation time 0 in this experiment, and sample subcultures were withdrawn at the generation time indicated Each sample was induced by the addition of 1 0 mM isopropyl-β-D-thiogalactopyranoside for 30 min, thoroughly washed on a Millipore filter and resuspended in buffer A Reducing buffer[11] (0 2 ml ml⁻¹) was added to each sample, which was then lysed with chloroform (0 02 ml ml⁻¹) After 20 min of agitation the samples were centrifuged at 4,000 r p m for 20 min, and the remaining chloroform was removed from the supernatant by evaporation with agitation at 37 °C (Samples lysed in a pressure cell gave identical kinetics of β-gal thermal denaturation) Portions (1 ml) of each sample were incubated in plastic tubes at 58 °C (stable to within ±0 05 °C) for various times determinedby varying the starting time The incubation was stopped by agitating all the tubes in 19 °C water for 1 min The tubes were then allowed to stand at 37 °C for 90 min before assay for β-gal activity by hydrolysis of orthonitrophenol-β-D-galactopyranoside at 37 °C (ref 12) The residual fraction of enzymatic activity is plotted as a function of the time at 58 °C The zero and 100-min points are each the mean of five samples The generation time for each curve is the mid-point of the sample induction period calculated from the history of absorbance readings (650 nm) taken from the main culture Repeated runs with improved resolution showed that, with this procedure, the decay kinetics of β-gal from untreated cultures is first order to within 2–3% for 200 min after the first 5 min *a*, Curves for samples out to 7 5 generation, *b*, the two subsequent samples with the 7 5 generation curve repeated Samples taken at the indicated number of generations (doublings) *a*, ○, 0, ▲, 1, ●, 4 1, △, 7 5 *b*, △, 7 5, ○, 11, ●, 16

effects Here we report the results of experiments with *Eschenchia coli* designed to detect evidence of self amplification of errors in protein synthesis If such error feedback is quantitatively significant, a slight increase in the pre-existing error rate should be followed by a progressive decrease in the accuracy of protein synthesis While this process could lead to a new stable level and thus need not precipitate a catastrophic breakdown, some late effects should be observable if the mechanisms are at all as hypothesised

We have manipulated the error rate in protein synthesis by exposing the cells to low levels of streptomycin, an aminoglycoside antibiotic which increases the rate of ribosome-mediated misincorporation of amino acids[9] The fidelity of protein synthesis in a culture at a given time was assayed by measuring the thermal decay kinetics of the enzyme β-galactosidase (β-gal) produced by a freshly divided and induced subculture The thousandfold inducibility of this enzyme permits a reasonably sharp definition of the time at which the accuracy of protein synthesis is measured

In the basic experiment in this series, a culture was sampled just before and at various times after addition of streptomycin at concentrations in the range 0 5 to 5 μg ml⁻¹ Typical results are shown in Fig 1 Before addition of streptomycin the β-gal thermal denaturation curve had reasonably first-order characteristics, indicating a correspondingly homogeneous enzyme population After addition of the drug a progressively increasing fraction of the newly synthesised β-galactosidase molecules showed enhanced thermolability

Simultaneously with these changes in the β-gal thermolability pattern, the cultures themselves showed a delayed, progressively decreasing growth rate and a progressive increase in the production of multinucleate, filamentous cells Recovery of the culture, as measured by thermal decay of β-gal, (Fig 1b) was also accompanied by increased growth rate and disappearance of elongated cells These observations support the idea that the thermal decay assay detects a specific consequence of a general cell-wide effect

A series of experiments similar to that depicted in Fig 1 lead us to several additional observations and tentative conclusions concerning this effect (1) Both the maximum extent and speed of development of the effect showed nonlinear, direct dependence on the concentration of streptomycin (2) Maximum extent of the effect was correlated with the speed of its development The smaller the effect, the longer it takes to develop, it was maximal in 8 to 10 generations for high drug concentration (2–5 μg ml⁻¹) and in 14 to 17 generations for low concentration (about 1 μg ml⁻¹) These observations seem inconsistent with the idea that the simple replacement of some cell component by its post-treatment equivalent (without feedback) is responsible for the progressive nature of the effect (3) The effect was sensitive to the growth medium and growth history of the culture (4) Within a fourfold range of growth rates (produced by varying the growth medium), generation time and not the actual time seemed to determine the kinetics of development (5) With treatments mild enough to allow growth for a few generations after addition of streptomycin, the cultures ultimately recovered Recovery to normal growth rate and cell morphology was always correlated with decreased levels of thermolabile β-gal We have, however, never seen complete recovery in as many as 25 generations after addition of the drug

We do not know whether recovery was due to adaptation within the cells or to selection of some subtly resistant variants If a favoured phenotype was being selected, however, it must have been present initially at a concentration of about 1 in 10³ The recovered populations are not resistant to streptomycin at levels of 20 μg ml⁻¹ or higher, hence, classical streptomycin-resistant alleles of the *strA* locus are not involved

Since we observed increased heterogeneity of the thermal decay rates for β-gal, it seems unlikely that the change was due to some other factor produced by the treated cells, which persisted through our washing, resuspension and lysis proce-

dures and enhanced the thermolability of the enzyme To che this possibility, however, we mixed lysates from induced b untreated cells in various ratios with extracts from late, dru treated cultures that contained only basal levels of β-gal Tl thermolability of the enzyme was unaffected even when tl extract from treated cells was in fivefold excess and wh parallel induced samples of the treated cultures showed tl expected enhanced thermolability

A convincing test of the same question comes from a experiment designed to show whether the increased therm(ability is due to modification of the enzyme after synthes Since enzyme synthesised before addition of streptomyc remained unaffected (Fig 2), we tentatively attribute tl observed alteration of the protein to faulty protein synthesis

Another experiment showed that the growth of late strept(mycin-treated cultures was much more sensitive to temperatu than that of untreated cultures (Fig 3), implying that the molabile, but otherwise functional, protein required for growl is being produced in the treated cells
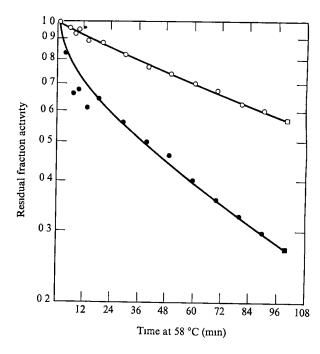
Fig. 2 Thermolability seems to be conferred at the time of protein synthesis Induced cells grown overnight to produce maximum levels of β-gal were deinduced by washing on a Millipore filter, and resuspended in medium containing streptomycin (2 μg ml⁻¹) A control sample was taken immediately (but not further induced) and the culture was allowed to grow for about 5 5 generations It was then divided into two samples (○ and ●), one of which (●) was induced for 30 min The procedures and conditions were otherwise as described in Fig 1 This figure shows the thermal decay curves resulting from these sample cultures The decay kinetics of the control sample were characteristic of untreated cultures and indistinguishable from the zero generation curve in Fig 1 and from curve ○ in this figure

If we were observing a progressive breakdown in the accuracy of protein synthesis, it could have been due to causes other than error feedback a slow increase in the uptake of streptomycin, possibly the result of a feedback cycle directly affecting permeation, or of some process involving the metabolism or binding of the drug Indirect evidence that such effects are not implicated came from our examination of the immediate effect of high doses of streptomycin on the production of thermolabile β-gal (data not shown) Substantial fractions of thermolabile enzyme were produced as an immediate response
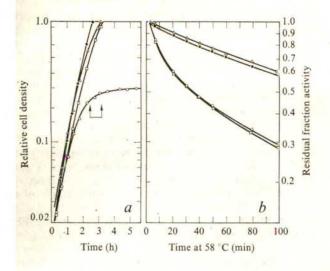
Fig. 3 Thermal sensitivity of the growth of treated cultures. Two freshly divided cultures of IH79, one with and one without streptomycin (2 µg ml^{-1}) were grown at 37 °C for four generations in exponential growth medium. At time zero, each culture was diluted into two flasks of the same medium, one held at 37 °C and the other at 42.5 °C. During subsequent incubation with agitation at these temperatures the growth of the cultures was measured by reading the absorbance at 650 nm. At the time indicated by the first arrow (*a*), samples were removed and induced at 37 °C. Induction was stopped at the time of the second arrow and the thermal degradation kinetics of the β-gal in each sample determined (*b*). Procedures and conditions were otherwise as described in the legend to Fig. 1. The strain IH79 normally grows well following a temperature upshift from 37 °C to 43 °C, but stops abruptly if shifted to 45 °C. ○, 42.5 °C, +streptomycin (2 µg ml^{-1}); △, 42.5 °C, −streptomycin; □, 37 °C, +streptomycin (2 µg ml^{-1}); ●, 37 °C, +streptomycin.

streptomycin and limitations of permeability of the drug revealed by pretreatment with EDTA–Tris[13]) played some role. The maximum immediate effect was, however, significantly less than the maximum late effect caused by streptomycin at µg ml^{-1}, and the high concentrations required ($\gtrsim$ 30 µg ml^{-1}) caused rapid cell killing. It therefore seems unlikely that the observed progressive effects of streptomycin resulted from changes in the intracellular concentration of active drug.

Mutations that confer resistance to high concentrations of streptomycin are mapped in the gene (*strA*) coding for a 30S subunit ribosomal protein (S12) and depress both the *in vitro* and *in vivo* error-producing effects of streptomycin[9]. Two resistant derivatives of IH79 and two independent resistant (*strA*) strains were found to have normal β-gal thermolability at concentrations of drug below 100 µg ml^{-1} but to produce a small amount of thermolabile enzyme (20% or less) when treated with 200 µg ml^{-1}. We conclude that the *strA* locus mediates the production by streptomycin of the progressive effect.

The results reported here suggest that error feedback in gene expression exists in *E. coli*, and constitutes a basis for further investigation of the stability of information transfer in gene expression.

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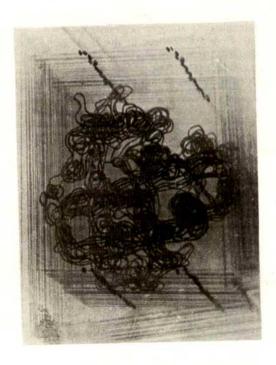
Structure of leghaemoglobin from lupin root nodules at 5 Å resolution

THE similarity in tertiary structure of mammalian haemoglobins[1-3] and myoglobins[4,5] and of haemoglobins from invertebrates[6,7] shown by X-ray analysis has been particularly important for our understanding of the biological evolution of these proteins. We have therefore studied the structure of leghaemoglobins—plant haemoglobins found in root nodules of leguminous plants—and compared the structures of seemingly unrelated, in terms of evolution, haemoglobins.

Leghaemoglobin from root nodules of the yellow lupin, *Lupinus luteus* L. is a monomeric haemoglobin of molecular weight about 16,000. The amino acid sequence for one component of leghaemoglobin from soya[8] suggested a correlation in structural organisation of the leghaemoglobin and other haemoglobins. The physiological role of leghaemoglobin is concerned in some way with the fixation of atmospheric nitrogen, formed symbiotically in root nodules of a higher plant by the bacteria *Rhizobium*.

Leghaemoglobin was extracted from root nodules by chromatography on Al_2O_3 and DEAE cellulose (*p*H 7.0), the protein being eluted by the ammonium acetate buffer. Crystals reached a size of 0.5 mm × 0.5 mm × 1 mm when grown in 28–33% saturated solutions of ammonium sulphate, buffered to *p*H 7.0 with 0.5 M ammonium acetate. The crystals are monoclinic (*a*=92.95 Å; *b*=38.31 Å; *c*=52.15 Å; γ=98° 50′; space

Fig. 1 The three-dimensional Fourier synthesis of electron density of leghaemoglobin.
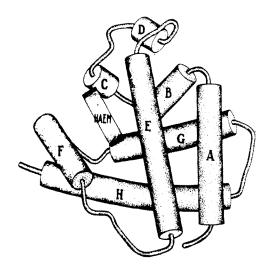
Fig. 2 The model of the leghaemoglobin molecule at 5 Å resolution.

group B2) and the unit cell contains four molecules, one per asymmetric unit[9].

The structure was determined using four heavy atom derivatives containing HgI_4^{2-}, UO_2^{2+}, I_2 and 3-HgCl–pyridine, respectively. The heavy atom positions in HgI_4^{2-} and UO_2^{2+} derivatives were obtained by difference Patterson syntheses, the other two were resolved using difference Fourier syntheses and protein phases derived from HgI_4^{2-} and UO_2^{2+} derivatives. The heavy atom parameters, refined using a Muirhead program algorithm[10], are listed in Table 1.

The best Fourier synthesis[11] of protein electron density at 5 Å resolution was calculated using 830 independent terms with the mean figure of merit 0.85. This revealed unambiguously the course of the polypeptide chain and the position of the haem group, with some indication of its orientation (Fig. 1). A very close resemblance between the tertiary structure of the leghaemoglobin and known animal haemoglobins and myoglobins was clearly shown. A similar myoglobin fold to that reported for sperm whale myoglobin[4,5] was found and the electron density map clearly shows the eight straight 'α helices' A, B, ..., H (Kendrew's nomenclature[12]) joined by rather short irregular segments.

Estimation of the number of residues in α helices based on the lengths of straight segments of high density gives, for A, B, E and G helices in the leghaemoglobin, numbers close to those for sperm whale myoglobin (16, 16, 20 and 19 residues, respectively). The F helix seems to be longer (about 14 residues as against 9) and the H helix is slightly shorter (23

residues as against 26). Similar differences were observed in tl haemoglobin from the marine annelid worm, *Glycera c branchiata*[13].

The haem group seems to be in the form of an oblate ellipsoi and can be distinguished by the highest value in the electro density map. As in other haemoglobins the haem position between E and F helices with the narrow side facing the Cl region. The haem plane is roughly normal to the line joinir those parts of the E and F helices nearest to the haem.

The model of the leghaemoglobin molecule is shown in Fi 2. The similarity in tertiary structure between leghaemoglob and animal haemoglobins (myoglobins) seems to have son interesting implications for both evolutionary and function aspects. If this similarity arises from a common origin of tl animal and plant haemoglobins it would mean that haem globin was present in organisms which lived about 1.3×10^9– 1.5×10^9 yr ago and in which the animal and plant kingdon had their origin. The tertiary structures which have arise during evolution therefore seem to have been conserve in widely divergent branches.

This similarity, however, parallels with the very poor corr lation of the primary structures of haemoglobins (in all know haemoglobin sequences the true invariants are only 3 out of total of 140–150 residues). It suggests that all the elements three-dimensional structure which have traversed such var ability in molecular chemistry unchanged, are indispensab not only for the stability of the molecular architecture but als for fulfilling their diverse functions.

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Table 1 Heavy-atom parameters

Compound	Site no.	Occupancy (electrons)	Fractional coordinates			B (Å²)	R*
			x	y	z		
K_2HgI_4	1	56.9	0.017	0.081	0	110	0.57
	2	35.8	0.214	0.404	0.132	86	
	1	39.5	0.189	0.512	0.592	70	
	2	26.4	0.123	0.098	0.545	70	
$UO_2(NO_3)_2$	3	16.8	0.241	0.966	0.669	70	0.52
	4	15.9	0.145	0.079	0.707	70	
	1	31.0	0.117	0.029	0.116	70	
I_2	2	29.4	0.159	0.133	0.155	70	0.53
3-HgCl–pyridine	1	49.5	0.200	0.614	0.462	70	0.62

$R^* = \Sigma(| \ |F_{PH}| \ - \ |F_P + f_H| \ |)/\Sigma(| \ |F_{PH}| \ - \ |F_P| \ |)$ calculated for all reflections, where F_{PH} is the structure factor for the derivative, F_P for the protein, and f_H for the heavy atoms.

Corrigendum

obituary

Hugh Blackwell Evans, the Antarctic pioneer, died at the age of 100.

Hugh Evans was born at Alvington, Gloucestershire, in 1874 and died at Vermilion, Alberta on 5 February 1975. In 1896–7 he went in the *Edward* on a sealing expedition to Kerguelen, acting as assistant to Robert Hall, the ornithologist. In 1898–1900 he was a member of the *Southern Cross* Antarctic expedition under C. E. Borchgrevink, and was the last remaining member of the first ten men to winter on the Antarctic continent. In February 1900 he was one of the four men who achieved the "farthest south" on the Ross Ice Barrier. He was assistant to the zoologist, Nicolai Hanson, and when he died Evans took over his work, receiving the congratulations of Dr. Bowdler Sharpe. In 1901 he was offered a place on the staff of the National Antarctic Expedition, but being in Canada, farming, he was unable to accept. He spent the remainder of his working life as a Prairie farmer. In the last couple of years he published three articles on his Antarctic experiences in the *Polar Record*.

Hyman Levy, the distinguished mathematician, died on February 27 at the age of 85.

Born in Edinburgh, Professor Levy was educated at the Universities of Edinburgh, Oxford and Gottingen. After four years on the staff of the National Physical Research Laboratory, he joined the Department of Mathematics at Imperial College, London, in 1920 as assistant professor, becoming professor in 1923 and head of the department in 1946 before retiring in 1954. The governing body of the college elected him one of its members and appointed him Dean of the Royal College of Science (one of the constituent parts of Imperial College) in 1946, a post which he held for six years. In 1957, he was awarded a fellowship by the college. Levy's most significant contributions to mathematics, and the development of mathematical teaching, arose from his interests in numerical methods. This, and the related subject of statistics, remained his favourite field of mathematics throughout his life. He was also deeply interested in Marxism and was a member of a British delegation Communist Party which visited Russia in 1956. He investigated reports that Jewish writers, artists and intellectuals had been persecuted, and apalled by his findings, he wrote an exposure of such persecution in the Communist weekly *World News* in January, 1957. He later became very much disenamoured with Marxism.

announcements

Awards

J. E. Hyde and **S. E. Reynolds** have been awarded Harkness Fellowships of the Commonwealth Fund.

The Institute of Physics has made the following awards for various contributions: **Guthrie Medal and Prize** to **D. Tabor** (study of surfaces); **Glazebrook Medal and Prize** to **W. C. Marshall** (direction of the work of the Atomic Energy Authority, particularly the administration of Harwell); **Charles Chree Medal and Prize** to **R. Hide** (hydrodynamics of rotating fluids and their applications to the major planets); **Thomas Young Medal and Prize** to **D. J. Bradley** (laser physics, particularly the tunable dye laser); **Duddell Medal and Prize** to **E. Ruska** (electron microscopy and electron optics); **Charles Vernon Boys Prize** to **R. A. Stradling** (electronic properties of semiconducting and semimetallic solids, particularly quantum transport phenomena and magnetophonon spectroscopy); **Maxwell Medal and Prize** to **A. J. Leggett** (behaviour of condensed matter at very low temperatures, particularly the elucidation of the transition in liquid ³He);

Bragg Medal and Prize to **W. A. Coates** (design and execution of demonstrations for lectures at the Royal Institution).

Sir Brian Flowers, FRS, has been awarded the **Chevalier de la Legion d'Honneur** for contributions to greater cooperation in the field of science between France and Great Britain.

R. M. Shackleton, FRS, has been awarded the **Clough Medal** for contributions to Scottish geology.

J. McManus has been awarded the **Clough Award** for work on modern sediments, particularly those of the Tay estuary.

Appointments

J. M. Hirst has been appointed Director of the Long Ashton Research Station, and to a chair and the Headship of the Department of Agriculture and Horticulture in the University of Bristol.

R. E. Richards will be the next Vice-Chancellor of the University of Oxford.

T. S. West has been appointed Director of the Macaulay Institute for Soil Research, Aberdeen.

R. Miledi, FRS, has been appointed Foulerton Research Professor by the Royal Society. Miledi is professor of biophysics at University College, London, and will continue his studies on nerve–muscle interaction there.

A Royal Society Research Professorship has been awarded to **J. W. Cornforth, CBE, FRS.** Cornforth is a director of the Milstead Laboratory of chemical enzymology at Shell Research and will take up his new appointment in the school of molecular sciences at the University of Sussex.

Miscellaneous

Developing Countries. The International Union of Pure and Applied Physics is to support the expenses of three scientists from developing countries during the Fifth International Conference on Atomic Masses and Fundamental Constants (AMCO–5), to be held in Paris from June 2–6, 1975. Applications (with curriculum vitae

and a written recommendation) to: AMCO–5 Secretariat, Institut d'Electronique Fondamentale, Bât. 220 Université Paris-Sud F-91405, Orsay, France.

Achievement Award. Applications are invited for this award, made annually for a British or Commonwealth achievement in the field of scientific instruments—research, design, or new technique—by the Worshipful Company of Scientific Instrument Makers. Applications (by March 31) to: The Clerk, Worshipful Company of Scientific Instrument Makers, Alliance House, 12 Caxton Street, London SW1, UK.

Essay Competition. The Bertrand Russell Memorial Logic Conference is offering a prize of £200 for an essay that examines in detail some aspect of the relationship between mathematics and the development of social or economic conditions. To be of general interest to mathematicians and include a consideration of current mathematical practice. Deadline: February 1, 1976. Further details from: Dr A. Slomson, School of Mathematics, The University, Leeds LS2 9JT, UK.

Foulkes Foundation. Offers a number of fellowships to science or other suitable graduates who have recently received a Ph.D. or equivalent, and who wish to take a full second degree course in medicine. Also for recently qualified medical men and women wishing to take a scientific, technological or other suitable subject. Applications to: D. W. FitzSimons, Secretary, Foulkes Foundation Fellowship, The CIBA Foundation, 41 Portland Place, London W1N 4BN, UK.

International meeting

April 15–16, **Understanding Energy Systems**, London (R. A. Davies, Operational Research Society, Sixth Floor, Neville House, Waterloo Street, Birmingham B2 5TX, UK).

Reports and publications

Great Britain

Theoretical Chemistry: Past and Future. By Professor C. A. Coulson. (An Inaugural Lecture delivered before the University of Oxford on 13 February 1973.) Pp. 38. (Oxford: Clarendon Press, London: Oxford University Press, 1974.) 50p net. [211

University College of Wales, Aberystwyth. Report of the Welsh Plant Breeding Station for 1973. Pp. 133. (Plas Gogerddan, near Aberystwyth: Welsh Plant Breeding Station, 1974.) 50p. [211

Conservation in Museums and Galleries: a Survey of Facilities in the United Kingdom. Pp. 115. (London: United Kingdom Group, International Institute for Conservation of Historic and Artistic Works, 608 Grand Buildings, Trafalgar Square, 1974.) £1. [221

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 277, No. 1270: A Discussion on the Origin of the Cosmic Radiation. Organized by G. D. Rochester, and A. W. Wolfendale. Pp. 317–501 + plates 12 and 13. UK £6.85; Overseas £7.05. Vol. 277, No. 1271: The Theory, Manufacture, Structure and Performance of N. P. L. X-ray Gratings. By A. Franks, K. Lindsey, J. M. Bennett, R. J. Speer, D. Turner, and D. J. Hunt. Pp. 503–543 + plates 16–19. UK £1.90; Overseas £2. (London: The Royal Society, 1975.) [231

Person to Person

Weinberg's Differential Rule. Additional data required to test this rule. Would workers routinely ascertaining and blood-typing twins at birth please let me have the following data: among the pairs known to be DZ on basis of blood groups alone, how many are same-sexed, and how many are opposite-sexed? (Dr W. H. James, Galton Laboratory, Department of Human Genetics and Biometry, University College, London NW1 2HE, UK).

Enzymes. A summer course is being organised by the Massachusetts Institute of Technology on 'Enzymes and their Use in Analysis and Clinical Diagnosis', from June 23–27. Aims to develop ability to use enzymes as analytical reagents and measure their activities. Director of the Summer Session, Room E19-356, MIT, Cambridge, Massachusets 02139).

Echange de Maisons. Famille à Londres veut faire une échange de maisons avec famille française, août 9–16. Offert: maison de 4/5 chambres, 4 étages, tout ce qu'il faut pour bébés/enfants; location paisible dans un faubourg agréable, près de Greenwich, 20 minutes par le train au centre de Londres; visites interessantes aux environs. Demandé: maison, ville ou campagne, endroit agréable, Bretagne, Paris ou le nordouest, pour famille de 3 enfants (D. Davies, 32 St Margaret's Passage, London, S.E.13, UK).

Mitochondrial Structure and Function. This course, sponsored by the University Board of Studies in Biochemistry, will be held from April 7–10 and is designed for postgraduate or other qualified students in the London area. Consists of a series of lectures and practical demonstrations on mitochondrial bioenergetics. Registration fee: £10 (Dr P. J. Quinn, Department of Biochemistry, Chelsea College, Manresa Road, London SW3 6LX, UK).

House Exchange. House sought in English or Scottish fishing village, fortnight August, preferably near the spring tides. Offer in exchange our house in Stratford-upon-Avon. Family of two adults, two boys (both primary age), (R. C. Hardwick, 3 Mayfield Avenue, Stratford-upon-Avon, Warwickshire, CV37 6XB, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements, and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Other countries

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Why EPIC should not be squeezed out

DURING April the Science Research Council (SRC) will have to announce some sort of a decision on the future of proposals to build a new high energy physics facility at the Rutherford Laboratory. The machine under consideration is a 14 + 14 GeV electron–positron intersecting complex called EPIC, and would replace the 8 GeV proton accelerator NIMROD and the 5 GeV electron accelerator NINA—Britain's present major machines. Support for and participation in CERN at Geneva would not be affected by the EPIC proposals.

Almost all accelerators at present in operation generate particles which are fired at stationary targets. As a result of conservation of momentum and of special relativity, most of the energy is wasted in propelling the products of the collision forward. A 400 GeV proton, for instance, fired at a stationary proton provides only 28 GeV of useful energy to the centre-of-mass system. This somewhat underpublicised characteristic of conventional accelerators becomes even less attractive when electrons are the projectiles, and least attractive when electrons are fired at electrons (or positrons). To produce the same 28 GeV in the centre-of-mass system of a conventional electron–positron machine would require 800,000 GeV! Hence high energy physicists have increasingly turned to colliding-beam systems in which two beams of particles travel in opposite direction around a ring and collide at regular intervals. The observer in the laboratory frame of reference then gets full value for money.

But why study electron–positron interactions at all? The scientist not conversant with high energy physics might with some justification ask in an innocent sort of way whether everything hadn't already been thrown at everything else, judging by the great bulk of *Physical Review* and the extraordinary sameness of the titles of papers therein. Apparently not; the more work that is done on protons, for instance, the more complex their substructure seems to be and the more inappropriate proton–proton experiments become for the elucidation of that structure. "It is rather like throwing two watches at each other in order to discover what their internal layout is" was how one high energy physicist described such experiments. But if the trend is then towards using simple particles (electrons are still mercifully believed to be point-like) as probes of complex particles, much more has to be learned about the forces on these simple particles—notably electromagnetic and weak interactions.

The scientific case for EPIC looks good, but scientific cases are hardly the only ingredient at present in decision making in science, particularly where the subject in question is indisputably a 'big science'. The Advisory Board for the Research Councils (ABRC) has recently warned that big science is in for a relatively lean time, and thus the EPIC proposal, which would involve 2,000 man years of staff effort and a capital investment of £25 million to get into operation by 1980, looks particularly vulnerable.

It would obviously make a positive decision much easier if the international dimension were more clearly known. Are there prospects for bilateral or multilateral deals involving cost sharing? It is difficult to assess this at the moment, as other countries, particularly Germany, are also interested in EPIC-type machines, and it is probable that if Britain says no, Germany will build.

The omens are not good. EPIC will be coming up for consideration at the same time as the Northern Hemisphere Observatory proposal; more and more often those involved in big science will find themselves in competition for limited money. It would be easy, and wrong, for the ABRC (which is likely to make the decision) to adopt a Buggins' turn attitude and let the astronomers have their observatory at the expense of EPIC because the astronomers didn't get their last big request, the Mark VA radiotelescope.

It would be equally wrong for the decision to be made in an atmosphere of antipathy to big science in which requests for large sums of money were rejected on some sort of principle that very fine work is being done by people who do not make inordinate demands on resources and it is time to cut the big boys down to size.

Finally, it would be wrong to turn down EPIC on the basis that high energy physicists are an isolated elitist bunch of people who have had a good run for their money, who don't do anything that anyone else can understand and who aren't within sight of any success which is going to provide benefits for the world-at-large. There is some force to these criticisms; high energy physicists are increasingly aware of the alienation between themselves and other scientists and of their general failure to have communicated the intellectual excitement of the subject to a broad audience. Even so, it would be grasping at a convenient but not relevant excuse to make a major decision for this reason.

Morale in the scientific community has never been lower, as job prospects diminish, governmental interest in science touches rock bottom and students turn away from science as a career. A very necessary step to restore some confidence is investment in large-scale long-term projects, and EPIC is just such a one. High energy physics shows strong signs of moving into a period of great excitement, and Britain's previous investments mean that there is much expertise in the field. Support for EPIC would show in an unambiguous way that those who make decisions in British science are aware of the need to restore confidence in pure science as an intellectual exercise, and are prepared to fight for it in high places. □
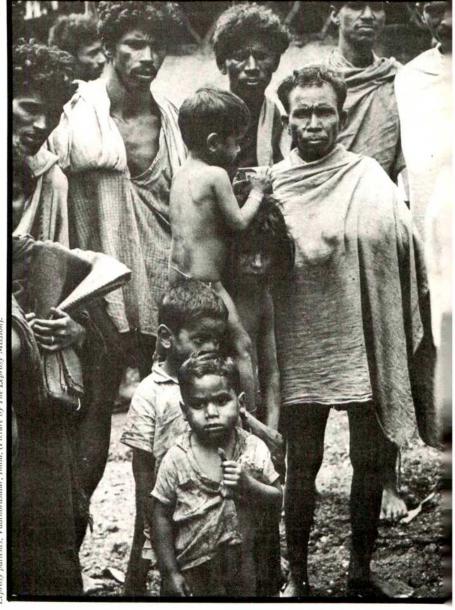
Leprosy: is the strategy wrong?

Authoritative opinion claims that there has been a failure to provide treatment for most of the world leprosy patients and to control the spread of the disease. But sulphones provide a cheap and practical form of treatment and also prevent transmission of leprosy, writes C. L. Crawford, of the Department of Anatomy and Embryology, University College, London.

IN 1873 the Norwegian Armaeur Hansen found bacteria in the tissues of leprosy patients and thus established the infectious nature of the disease. Today the World Health Organisation (WHO) estimates there are 10.8 million people with the disease, of whom only about 18% are receiving any treatment. Although information is limited it also concludes (*Bull. Wld. Hlth. Org.,* **46,** 523; 1972) that there has not been any improvement in controlling the spread of the disease over the past five years. The British Leprosy Relief Association (LEPRA) claim that there are as many as 20 million patients. Browne and Davey, two of the most experienced leprologists, (writing this year in *Leprosy Review*), conclude that existing methods have failed to reduce the incidence of the disease, and Ambrose *Nature,* **248,** 370; 1974) states that leprosy is increasing in South Asia.

Leprologists stress the unknown facts about the disease; the bacteria cannot be cultivated so a vaccine cannot be developed, and the mode of transmission and the incubation period are unknown. Faced with this lack of knowledge and the pessimism of authoritative opinion, the outlook seems hopeless both for success in preventing the spread of the disease and for the patient's chances of obtaining treatment. But the efficacy of the sulphone in providing a cheap and practical form of treatment and in prevention has been grossly neglected.

The difficulty in culturing *Mycobacterium leprae* could, in fact, be seen as a potential advantage in an attack on the disease. *Mycobacterium leprae* is confined to humans and must therefore exist outside the body only for very short periods. There is no known animal reservoir of infection or vector to spread the disease and transmission takes place only between humans. Probably only a proportion of leprosy patients are responsible for

Leprosy patients, Vadhiorasalur, India. (Picture by The Leprosy Mission).

transmitting the disease. This multi-bacillary or lepromatous group may be as low as 10% in some parts of the world and constitutes the reservoir of infection. Also only one in ten of the exposed population, at the most, acquire the disease even in the absence of control measures. If transmission could be interrupted in susceptible people, the disease would decline, as indeed it did in Norway in the last century by a policy of segregation. Also, once eradicated from any country it has never returned. A vaccine is not essential for eradication as the disease has already disappeared from many countries. The transmission of yaws has been successfully interrupted without the bacteria being cultivated or a vaccine developed, by treating patients with penicillin.

The sulphones are the main drugs used in the treatment of leprosy. The experimental multiplication of *M. leprae* in mice is inhibited if *M. leprae* from patients who have received more

than three months of sulphone treatment are injected, suggesting that such bacteria are no longer viable. It may thus be that patients with leprosy are no longer infectious after a few months of treatment, and sulphone may have an important role in preventing the spread of the disease as well as in treatment. Sulphones have been used for the past 25 years and it is essential to determine if they have had an effect in preventing transmission of leprosy.

Any successful preventative measure against leprosy will be shown by a fall in the number of new cases or in the incidence rate; the total number of cases (or 'prevalence' rate) will change much more slowly, because of the inclusion of patients who are already crippled. If the majority are of this type, the prevalence may hardly change at all over a period of years even though transmission may have been completely interrupted, because leprosy patients can live a long time

The life expectancy of untreated lepromatous leprosy in Hansen's time was about 10 years; now, with sulphones, it is the same as in the general population. Thus, the 'prevalence' may actually rise in a community treated with sulphones. It is therefore very surprising that the WHO should attempt to draw conclusions about the spread of the disease from the figure of 10.8 million, which is a prevalence estimate. No attempt has been made to obtain information about incidence rates, and even the estimate of the total number of cases is based on very questionable assumptions. Of the 10.8 million, 2.5 million are actually registered; 0.5 million of these are cured or arrested. In each country, to make allowance for unknown cases, a percentage of the registered cases was calculated depending on the efficiency of case finding. This varied between 25% and 300% of the registered cases. This total was added to the 2.5 million registered cases to make up the total of 10.8 million. There must, however, be doubts about the value of figures arrived in such a haphazard way. Where accurate figures of leprosy patients are available, as in Rumania, Cyprus and Libya, it has been shown that the WHO has exaggerated the true figure by from 10 to 50 times. In commenting on the WHO errors in the Cyprus figures, the Cyprus Director-General of Health emphasised that all the patients were old ones and no new cases had occurred in 1970, thus confirming the danger of drawing conclusions about the spread of the disease from prevalence figures. Countries which do have information about incidence rates, such as Okinawa, Hong Kong, New Guinea, Japan and Solomon Islands, Rumania and Cyprus, have all shown a decline. Although their figures will not be completely accurate, they are preferable to the WHO method of assessing the problem.

Most of the countries mentioned have a small leprosy problem. The most accurate information about incidence rates in highly endemic areas has come about in the following way. As no leprosy vaccine is available, BCG vaccine against the similar tubercle bacillus has been used to determine whether it has a protective effect againt leprosy. Trials have taken place in Uganda, Burma and New Guinea under the auspices of various research councils, and two trials have also been carried out in India using sulphones for their protective or chemoprophylactic effect by giving them to healthy people. All the trials were controlled by including a group similar in age range which was not protected with BCG or sulphones. At the start of trials the population was sur-

veyed, the leprosy cases were excluded and then new cases were recorded at repeated intervals of between one and two years. Thus, an annual incidence rate can be deduced and the control group will reveal the trend of the disease in these areas, which were deliberately chosen because of the serious leprosy problem.

The results of the BCG trial in Uganda and the two chemoprophylactic trials in India are shown in Tables 1 and 2. All the control groups shows a striking decline in the incidence of leprosy. These two trials took place in communities where sulphones are available to treat patients and, in contrast, the incidence of leprosy in New Guinea did not decline, staying constant at 6 per 1,000 per year from 1964 to 1969; sulphones were not available in this area until late in 1967. Therefore, the most likely explanation of the declining incidence rates is that sulphones given to leprosy patients kill *M. leprae* in a short enough time to interrupt transmission of the disease, thus confirming the experimental findings. The figures for new lepromatous cases are even more dramatic. In two of the areas not a single case was recorded in the controls and in the third the number fell from 11 to 2. Thus, the reservoir of infection has been virtually removed and this makes prospects of eradication bright. The chemotherapeutic and BCG trials were started over ten years ago yet delay in publishing the results fully and in coming to a conclusion about their efficacy has meant that a really effective method of prevention and eradication with sulphones is not being put into practice. If there is still any doubt about the conclusions to be drawn from these trials, these areas

could be resurveyed and the incidence rates recorded in the same age groups as those of ten years ago. There is other evidence based on outpatient incidence rates from two areas of India, Zambia and Uganda and from prevalence rates over a long period in Northern Nigeria, and former French Equatorial Africa (Gabon, Chad, Central African Republic and Congo-Brazzaville). All these have been summarised with my conclusions of the BCG and chemoprophylactic trials previously (*Lancet*, ii 1375, 1971; i, 1186, 1972; *Trop. Doct.*, **3**, 137, 1973). Also there is recent evidence of a decline in incidence in Burma and Thailand. Yet Ambrose, Davey and Browne have not mentioned these nor produced any quantitative information to support their own case.

If chemotherapy with sulphones does prevent the spread of the disease then treatment and prevention can be achieved by the same measure, the administration of the drugs to leprosy patients, especially those who are bacteriologically positive. Also by offering to the patients an incentive to attend for treatment, the affected group in the community is automatically brought under supervision, an immense practical advantage over other methods of prevention such as BCG and chemoprophylaxis, which depend on finding healthy contacts of patients. The main sulphone is dapsone which is extremely cheap, costing only 10p a year for each patient, and is easily administered by mouth once a week. Drug resistance is very rare, of the order of 3 per 5,000 lepromatous patients, and it has a wide margin of safety for toxic effects. Thus, mass treatment is practical and economically feasible if given in villages by

Table 1 Annual incidence of leprosy per 1,000 in control and BCG groups in the BCG leprosy trial in Uganda

	Control			Protected with BCG		
	Population examined	No. with leprosy	Annual incidence	Population examined	No. with leprosy	Annual incidence
1964	8,071	89	5.5	8,091	18	1.1
1966	9,036	54	3.0	9,052	1	0.05
1968	9,036	31	1.7	9,052	8	0.45

The incidence rate has been halved to give the annual incidence as there is an interval of two years between each survey.

Table 2 Annual incidence of leprosy per 1,000 in Andra Pradesh, India, in controls and chemoprophylactic groups: *A*, under 25 years of age; *B*, over 25 years of age

	A					
	Controls			Protected with Sulphones (Dapsone)		
	Population examined	No. with leprosy	Annual incidence	Population examined	No. with leprosy	Annual incidence
1965	11,270	54	4.79	11,452	29	2.53
1966	12,124	65	5.36	11,900	14	1.17
1967	12,116	37	3.01	12,157	9	0.74
1968	12,931	36	2.78	12,754	3	0.24
	B					
	Controls			Protected with Sulphones (Dapsone)		
	Population examined	No. with leprosy	Annual incidence	Population examined	No. with leprosy	Annual incidence
1965	8,104	90	11.10	7,851	91	11.59
1966	7,758	67	8.63	7,758	62	7.95
1967	7,337	38	5.17	7,511	45	5.9
1968	7,311	29	3.9	7,282	34	4.6

auxiliary staff who have been trained in diagnosis and treatment.

One area where this approach has been developed most widely is in northern Nigeria and was initiated by Ross. He carried out surveys, trained auxiliary staff and set up outpatient clinics in villages. Patients accepted this approach enthusiastically, whereas before there had been a poor response to treatment based on the leprosarium. In one area, 639 new patients appeared for treatment within 3 months of opening the clinics. There was little fear of stigma against the disease and Ross comments: "There is a degree of shame attached to infection by the disease. Nevertheless, leprosy is tolerated in village and social life and any fear of detection has been associated with the dread of being segregated which means separation from one's family". By 1968 there were 2,000 outpatient clinics throughout the northern region and most patients had access to treatment at an early stage. Unfortunately, few countries adopted such an approach so comprehensively; in many the leprosarium remains the only place where treatment can be obtained, patients have to travel long distances and they fear segregation. The numbers on treatment have remained low for these reasons and not because of lack of money as LEPRA claims in its advertisements. The cost of maintaining patients in institutions is enormously high and measures such as reconstructive surgery have added to the financial burden, making even less money available for work in outpatient clinics. Also, diagnosis is late; patients come late in the course of the disease and thus many are crippled and hence do not benefit from dapsone treatment.

Voluntary agencies in Europe collect more than £2 million annually for leprosy work, but most is still spent on institutional care—in May 1973, for example, the *Sunday Times* published an illustrated article showing the plight of severely crippled leprosy patients in India. At the same time LEPRA appealed for money. £20,000 was collected and in a letter to the *Sunday Times* on December 13, 1973, the Director of LEPRA gave details of how this money was to be spent. All of it was to be used on institutional care, accommodation and food, and on enabling patients to learn a trade such as footwear and cloth making; little money will go to curing and preventing the disease. As most of the patients are already crippled they cannot benefit from dapsone treatment. While there is sympathy for these patients' needs, surely the bulk of the money should go to prevention and cure with sulphones, which LEPRA claims is its aim. □

Accounting for energy

Dr Malcolm Slesser, Director of the Energy Studies Unit, Strathclyde University, sets out the basic principles of energy analysis.

IF economics is the science of scarcity and substitution, energy analysis is no more than the application of economic theory to the one commodity in ultimate limitation on Earth—thermodynamic potential. Perhaps the greatest conceptual difference between economics, as we know it today, and energy analysis lies in the 'system boundary'. Economics sets up a boundary around the system of interest; in energy analysis the system boundary is the whole planet.

Within the framework of economic thought, the economic process will automatically stimulate the development of another energy resource as one resource is depleted and its marginal costs rise. In effect, economics treats the world as a closed system having access to limitless amounts of energy, whose acquisition takes only time, capital labour and technology. On the face of it, it is a good deal easier to justify the position of the economists than that of the energy analysists, but it can be looked at another way.

Any production process, such as building a road or manufacturing pig-iron can only succeed if we inject capital, labour, materials and energy. Considering the properties of these inputs, capital is no more than labour, materials, capital and energy invested at an earlier time. If one goes on to examine every one of the inputs to any process one finds that these inputs are themselves the outcome of a process involving capital, labour, materials and energy. Indeed, the process of network analysis can go back and back until we find that the materials are ores in the ground or produce on the land and the energy is hydrocarbon locked in the

Earth or in deuterium atoms in a highly dilute state in the oceans; labour is mankind and capital has disappeared.

Although both energy and materials are mined and utilised, they have very different features. Materials are never destroyed; the iron molecule in iron ore is still an iron molecule when it is turned into steel and when it ultimately ends up as rust. Moreover, there can never really be a shortage of ores, since the oceans contain very large quantities. We are, however, long past that stage of global development at which ores can be obtained at sufficient rates simply by men labouring with picks and shovels. The energy required to win increasingly depleted ores and turn them into concentrated form will rise to enormous values. It is not the scarcity of the ores that is the problem.

When this thinking is applied to the two examples of making pig-iron and road building, it is certainly conceivable that, given enough poor and willing citizens, a motorway could be constructed by human labour alone. But no amount of human sweat can turn iron ore into iron, or iron into sophisticated machine tools. Though there is a well understood marginal energy cost for labour, so that the two can substitute one for the other to some extent, in the last analysis, energy does what labour cannot do. It carries out processes of transformation involving a decrease in entropy. Energy is irretrievably degraded once it has been used. All energy finishes up as waste heat, and must eventually be dissipated to the surrounding space. Labour, by contrast, is renewable and can also be upgraded through such processes as education and training. Also, as development has proceeded, labour, except in its most skilled forms, has become a surplus commodity in the greater part of the world.

Accordingly, energy analysts believe that it makes sense to measure the cost of the things done, not in money, which is after all nothing more than a highly sophisticated value judgement, but in terms of thermodynamic potential. On the other hand it is widely recognised amongst energy analysts that, as R. S. Berry has stated so succinctly, "if economists in the market place were to determine their shortages by looking further and further into the future, these estimates would come closer and closer to the estimates made by their colleagues, the thermodynamicists".

As energy analysis is concerned with the amount of global energy stock which must be sequestered to make a good or service, it is not enough to assess the amount of oil consumed to heat a house. What is of interest is the total amount of oil in the ground, to-

gether with any other energy sources, that had to be used to make that house heating possible. Thus we also need to know the quantities of energy that were required to win the oil from the ground, bring it from the oil field, refine it and deliver it to the consumer. In the UK these processes add about 18% to the apparent energy requirement for fuel oil for home heating. The error in ignoring the energy requirements for supply are in this case small, because the direct energy costs are the major component. But if we apply the same error to UK agriculture, for example, the direct energy requirements are a mere 42% of the total amount of energy that must in the end have been sequestered in order to make UK agriculture possible. This will have included the energy to make the tractor (duly amortised), pesticides, fertilisers and so on, as well as an appropriate share of the energy to make the machines that make the fertilisers, tractors and so on.

Until August 1974, various practitioners of energy analysis were, of necessity, formulating their own rules. In that month the International Federation of Institutes of Advanced Study IFIAS) held a workshop in Sweden, attended by twenty people from nine countries, at which the methodology and conventions of energy analysis were thrashed out. Its report is now available (*Workshop Report No. 6,* IFIAS, Stockholm; £4), and the following remarks follow its recommendations.

Perhaps the most fundamental observation is that since our concern with energy is its ability to do work, the calorific or heating values of fuels are not really the quality of interest. Rather it is a more subtle thermodynamic quality known to engineers as available work' which in turn closely equals a more easily derivable thermodynamic property, the Gibbs free energy. And indeed in any refined study in energy analysis such as the assessment of waste, for instance, it is the free energy that should be measured.

Nevertheless, the members of the workshop recognised that the calculation of Free Energy would not always be possible and is in any event little understood. For most situations an error of no more than 10% is incurred in taking enthalpy, that is the heating value of a fuel when it combusts with This quantity is called the Gross Energy Requirement (GER), normally expressed in megajoules (MJ) or gigajoules (GJ) per unit of output. The workshop defined it as "the amount of energy source or sources which are sequestered by the process of making a good or service". To obtain the 'energy' content, the workshop recommended the convention that it be taken as the

gross heat of combustion with air at 1 bar pressure and O °C.

The ramifications of this concept may be gathered from Fig. 1, in which is depicted a hypothetical production process in which natural gas, feed-stock hydrogen and silicon dioxide (from sand) combine to yield a product Y. Suppose that the energy to drive the process comes from coal. There are three system boundaries, of which the inner may be likened to the factory fence, and is essentially the system boundary a d o p t e d by company accountants.

The inputs entering through the inner boundary have to be prepared and delivered. Thus silicon dioxide must be refined by (say) acid treatment of sand, hydrogen must be made from natural gas, and natural gas must be brought to the factory fence. These additional processes are depicted by the middle system boundary, somewhat analogous to the nation state. But in the end all the inputs derive from a source in or on the ground—the outer system boundary. In estimating the GER it is essential to go back to this level. Of course, if one's interest is in the energy efficiency of a given sector of a process, then one may choose to examine what happens in the transfer through one boundary to another. This is referred to as the Process Energy Requirement.

Another valuable permutation is that of Net Energy Requirement (NER).

Fig 1: hypothetical production process
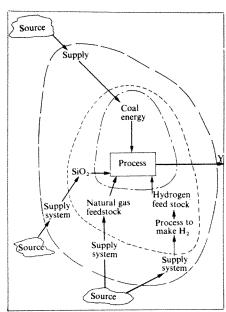
Table 1 The ERE for UK fuel industries

	1963	1968	1971-72
Coal	0.047	0.042	0.047
Gas	0.48	0.390	0.23
Oil	0.23	0.134	0.11
Electricity	3.54	3.192	2.98

Source: Chapman et al., Energy Policy, Sept. 1974.

This is useful in cases in which the product is itself a combustible item, such as a plastic product or a refined fuel. It takes into account the fact that the product has a future potential as a fuel. Clearly in the argument as to whether a plastic or a glass bottle is the less energy intensive, it aids the proponent of plastic bottles to take the NER, rather than the GER, while the manufacturer of the glass bottle cannot take anything but the GER.

Of all the uses of energy analysis and the use of Gross Energy Requirement, perhaps none is more spectacular than the concept of the Energy Requirement for Energy (ERE) defined as (energy of the resource sequestered—energy delivered to point of use)/energy delivered to point of use. Here we use energy analysis to assess the amount of energy that must be sequestered in order to make energy available. Table 1, for example, relates to some recent UK figures. It shows some facets of our energy industries that are not normally apparent. Thus the efficiency of fuel use for electricity generation is rapidly rising and now exceeds an average thermal efficiency of 31% at the power station. But if one allows for the energy required to get the coal and nuclear fuels to the power station, the energy dissipated in the grid and that used by the electricity boards themselves, the final ERE for electricity in the UK today is barely below 3.0. That is to say, for every unit of electricity consumed by the public (1 kWh = 3.6 MJ), some 14 MJ of energy have been sequestered.

Estimating energy requirements is not unlike cost accounting with an element of quantity surveying thrown in. One should recognise that various levels of accuracy are necessary. The direct inputs to a process under study must be estimated accurately. But the inputs to the inputs may generally be treated with a lower order of accuracy. Thus if the problem is to estimate the GER of a field of barley. one needs to know the actual inputs to the barley field. These will comprise such things as tractor fuel, nitrogen fertiliser, phosphorus, pesticide and so on. But the GER to make nitrogen fertiliser need not generally be estimated in such detail. Indeed an industry-wide aggregate based on the GER per unit of nitrogen would be sufficiently accurate. In the same way the machines to make the factories to make the inputs represent a very small part of the whole process, and may be obtained at adequate accuracy from such devices as economic input-output tables.

Perhaps the greatest room for dispute and error is in the partitioning of the GER between two or more products of one process. In economics, market forces dictate partitioning. In energy

analysis, the market mechanism has yet to develop. For example, how does one partition the energy requirements in the case of the electrolysis of common salt to yield chlorine and sodium hydroxide, for each of which the current price is different? The IFIAS workshop recommended that this be done on the basis of some physical parameter, not on notions of value.

Energy analysis has been cursorily dismissed in some economic circles as being no more than "a BTU-theory of value". Energy analysts, however, would neither claim as much nor as little. The potential for energy analysis still has to be tested, just indeed as economic analysis has still to show whether it is sufficiently developed to deal with such problems as resource scarcity or a step change in the price of energy. I shall merely indicate here a few of the areas where energy analysis is being tried out and suggest that it is a logical area of endeavour.

What will be the price of ammonia fertiliser in 1990? It would be a brave man indeed who was prepared to extrapolate price trends that far ahead. Even to guess at a figure for six months from now would have its uncertainties. By using energy terms, the uncertainty largely vanishes. Figure 2 depicts how the GER for ammonia has changed with time since its first synthesis in in 1912. Note how the GER appears to be flattening out at some 2.5 times the theoretical (thermodynamic) minimum. Now the thermodynamic minimum energy presupposes an infiinitely slow rate of transformation—clearly not a practical state of affairs for a world that is looking for large amounts of ammonia every day. It is now clear that there is a trade-off between energy and haste of production, and a study of the new, really large (300,000 tonnes a year) ammonia plants suggest that the technological minimum at that rate of production will be not far from the value of 2.5 times the thermodynamic minimum. This simply arises from the need to have preheaters, coolers, heat exchangers and furnaces that are less than 100% efficient.

We can therefore forecast with reasonable accuracy that the GER for ammonia in 1990 will be about 45 MJ kg^{-1}.

Where less developed processes are being dealt with, of course, an assessment must be made of the effect on the GER of technological improvements and growth in the scale of operations. Economic theory would have to be used to find a means of assessing how quickly these changes might come about under the pressures of demand and supply.

Since all energy is conserved, but high quality energy is degraded to waste heat, waste has several connotations.

In one sense it is the use of more energy than necessary to effect a given transformation. Energy analysis provides the tools for looking at this problem, though it does not of itself provide the answer. Gyftopolous and associates (in *Potential fuel effectiveness in industry*, Ballinger, Cambridge, Massachusetts, 1974) estimated the Gross Free Energy Requirement (GFER) in various production processes in the USA and found that iron making, at 4.16 times the thermodynamic minimum, was much more efficient than the aluminium industry (7.6), petrol (10) or paper making (170).

But there is another sense in which energy analysis can examine waste. It
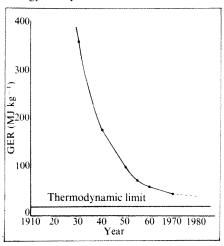
Fig 2: GER for ammonia manufacture

is now recognised that energy dissipation in highly industrialised areas can create climate modification and some climatologists are suggesting that, globally speaking, an energy dissipation equal to 1% of the incoming solar energy or even less may be an upper limit for the planet. As we approach this limit, probably in the middle of the next century, the matter of interest is not the energy dissipated by the factory in Sheffield, but the energy that had to be dissipated in order to make the Sheffield factory function. And that has to be aggregated over the whole globe. This is exactly what a GER calculation allows one to do so easily.

There are many views of the future energy situation and how the price of oil will vary. It is reasonable to argue that in intelligent hands (and OPEC is intelligent) the price of oil will be slightly lower than its substitution price. But economic substitution calculations based on pre-November 1973 energy prices are now incorrect, because the stepchange in the price of oil has raised the cost of the inputs necessary to furnish alternative forms of energy. So calculation of that substitution price is a very tricky matter. Why not re-express the problem in terms of ERE?

In 1972 it was reckoned that Colo-

rado shales would be exploitable at an energy price of $6 a barrel, that is when oil prices reached a price of $6 per barrel (US Energy Outlook, National Petroleum Council, USA). At that time energy cost somewhat less than $1 a gigajoule. Allowing for others costs and profit, we deduce that the GER for shale was and is around 5 GJ per barrel. The energy in a barrel of shale oil is about 6.5 GJ per barrel. Thus shale production gives a net ERA of about 0.3. King Feisal is still sitting pretty.

Such figures are clearly very tentative and not backed by good data. But the data are obtainable.

The ceiling to energy prices will be set when the ERE for unlimited energy is known. Our likely sources are fusion and solar. At the moment the ERE for solar energy by photovoltaic methods is around 2—hopelessly high. New technology in the pipeline may reduce that by a factor of six to 0.33—better than Colorado shale — but another order of magnitude is needed before solar energy sources can compete with Middle East oil at the well head. The ERE for fusion is a matter for conjecture. In laboratory studies it is still hopelessly high.

And so, finally, we come to the logical intermediate step—to assess the GER to save energy rather to produce it. What, for example, is the GER to double glaze and insulate a house, and what is the GER saved thereby each year? When energy was cheap, economic calculations came up with different solutions. Now they are tending to come to the same solutions and people, using their own horse sense, are insulating and double glazing their homes and are finding it an extremely attractive long term gain.

One of the most telling uses of energy analysis has been the pioneer work by Chapman and Mortimer at the Open University and by Price at Friends of the Earth in their dynamic analysis of nuclear reactor building programmes. They show that no crash programme of nuclear reactor construction can ever meet an energy gap, because of the huge energy investment needed; indeed a net energy deficit would be created for the first eight to eleven years of the programme. At Strathclyde we have carried this analysis a step further using system simulation techniques to examine a range of energy scenarios for the UK to the year 2 000. Though a great deal hinges on the quality of uranium ores that will be available, there seems to be no way a steam generating heavy water reactor system can sustain the rates of economic growth hoped for. Here then, is a new methodology, an old language and a familiar problem—the problem of allocation of resources. □

international news
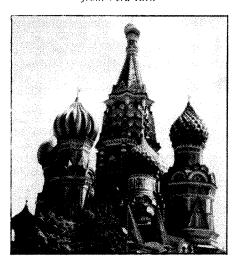
As the current five-year plan draws to its close, considerable concern is evident in the pronouncements of the Central Committee of the Communist Party of the Soviet Union (CPSU) about the lack of progress in the Soviet research and development programme. According to *Pravda* (February 11, 1975), the plans for implementing new technological advances have not been fulfilled in "a number of enterprises of the Ministry of Power Production, the Ministry of Ferrous Metals and the Ministry of the Coal Industry. In several teams at Institutes of Higher Education, regular work on pressing problems of science and the national economy has been replaced by vain bustle between petty and inefficient themes. The possibilities of mutual exchange of achievements between enterprises, institutes and branches are not being used to the full. ... In certain institutes up to 40% of the work completed has not been realised for years". The Central Committee stresses the need for the appropriate ideological means to be taken to "put into action all reserves for accelerating scientific and technological progress", in particular, the improvement of the organisation of "socialist emulation" among the scientists and technologists concerned.

● The announcement of a new *samizdat* journal, *Twentieth Century,* edited by Roy Medvedev (the twin brother of geneticist Zhores Medvedev, who was deprived of his passport in 1973) raises once again the question of academic dissidence in the Soviet Union. Although Roy Medvedev, together with physicist Valentin Turchin, was a co-signatory of Sakharov's second "Manifesto" (1970), over the past few years there has been an increasing gap between the brands of dissidence expressed by Roy Medvedev and Sakharov. The new journal is a successor to the *samizdat Political Diary* of the late 1960s, which took, in the main, a Marxist viewpoint, although critical of the Kremlin's interpretation of Marxist–Leninist principles. It would seem that Roy Medvedev and his contributors envisage *Twentieth Century* as an alternative press for the loyal opposition—a halfway house between the official establishment journals and the more outspoken dissidence of the *Chronicle of Current Events.* The latter reports cases of breaches of constitutional

rights and the fate of prisoners of conscience, as well as discussing general issues. On the other hand, *Twentieth Century,* to judge from the contents of the first issue, seems likely to concern itself primarily, if not entirely, with theoretical debate.

Meanwhile, at the other end of the spectrum of dissidence, the appeal of Dr Mikhail Shtern against his eight-year sentence still remains postponed and unheard. His son, Avgust, who re-

Russia today

from Vera Rich

cently arrived in Israel, connects the sudden granting of his visa with his father's predicament. It is, he says, more "convenient" for the prosecuting authorities that his father should be alone, without the support of his family; it seems, in fact, that his own visa was granted on an 'either/or' basis, which left Avgust little choice but to depart for Israel. He stressed that his father is now in a very precarious state of health and that, under prison conditions, the after-effects of an old injury from a car accident could at any time produce paralysis. In his concern for his father, Avgust seemed unwilling to discuss the details of the accusation, except to state that his father has not broken or contravened any Soviet laws. He stressed, however, that Dr Shtern's chances of surviving an eight-year sentence are minimal and that only a successful outcome of the appeal can save him.

One dissident who has survived his term of imprisonment is Ilya Glezer, a neurobiologist, who was sentenced in

1972 to three years in a labour camp and three years "exile" (in Siberia). A massive appeal (151 signatures) was organised on Glezer's behalf by scientists and physicians working in related fields, asking that in the interests of international good-will he should be exiled not to Siberia but to Israel. Nothing, however, has been heard of Glezer's whereabouts since the expiration of his term of imprisonment on February 7, and it would seem therefore that the appeal has been ignored or denied.

● The Lomonosov medals for 1974 were awarded to Academician Aleksandr I. Tselikov for his work in metallurgy and metal technology, and to Angel Balevskii, President of the Bulgarian Academy of Sciences, also for his work in metallurgy. The Lomonosov medals are the highest awards of the Academy of Sciences of the USSR; two are awarded annually, one to a Soviet and one to a foreign scientist.

● The *Novosti* agency reports that a team of Leningrad scientists has developed a computer-controlled robot with laser "eyes" and a crab-like walking motion. According to the team spokesman, Mikhail Ignat'ev, it has high cross-country ability with minimum energy consumption, and is several times cheaper than a comparable air-cushion craft. It can automatically set itself to proceed at any speed from a walking pace to a "gallop", and can lift loads of up to half a ton. Ignat'ev suggests that it could be of great use in enabling geologists to cross difficult terrain—dense forests and steep mountain slopes. The team is now at work on a model which will not run down any saplings growing in its path, thereby conforming to conservation requirements.

● The February 1975 issue of the popular science journal *Priroda* carries a headline in its news section that at first glance seems an ominous echo of Lysenkoism—"Hybrid of a herb and a tree". In fact, however, the hybridisation reported is simply that of two species of *Nicotiana*—the dendroid smoking tobacco *N. glauca* and the flowering *N. alata* used in horticulture.

The research, which was carried out at the Principal Botanical Garden of the Soviet Academy of Sciences rseulted first of all in sterile hybrids which were then, it is claimed, converted by "colchicinisation, leading to doubling of the number of chromo-

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somes and normalisation of the formation of sex cells" into fertile amphidiploids. It is hoped that the hybrid will form "interesting material" for the selection of disease-resistant strains of smoking tobacco—a revealing sidelight on Soviet public health planning, which, although greatly concerned with the fight against alcoholism, shows no signs of following this up with an anti-smoking campaign.

● Examination of the photographs of the Sun from the Salyut-4 mission have revealed considerable differences in the clarity of pictures taken by the same telescope, even on the same day. Although for the mission due allowance was made for the possibility of the condensation of fine particles on the optical elements, and the reflecting surface was deposited on the mirror immediately before observations were to begin, it would seem that this was not enough. In one case, one of two photographs taken only a short time apart shows a distinct image with good contrast, whereas in the other the Sun is "hardly" visible.

The Soviet space programme has always been considerably automata-oriented—the early cosmonauts being little more than human test animals in their craft. A brief note in *Pravda* on the quality of the photographs is, therefore, perhaps, of more than passing interest. After reporting that the scientists of the Crimean Astrophysical Observatory of the Soviet Academy of Sciences are now engaged in evaluating the photographs and in relating them to the spectrograms obtained with special reference to protuberances and flocculae, it wryly remarks that the scientists hope that the cosmonauts who actualy operated the orbital telescope may be of "great help" in this task.   □

Energy costing in the fields

by Eleanor Lawrence

THE Agricultural Research Council has recently made public the results of a Working Party on energy in agriculture which was commissioned by the relatively new Joint Consultative Organisation for Research and Development in Agriculture and Food. The report was intended to provide an appraisal of the use of energy in British agriculture and recommend ways in which agriculture could use this energy more efficiently while continuing to increase its productivity.

According to the overall energy budget calculated in the report, British agriculture at present uses only 3% of the annual national energy requirement to produce just over 50% (on the basis of cash value) of the nation's

food. Impressive as this figure is, it is difficult to find out from the report whether in fact this 3% is being used as efficiently as it might be, as ultimately the energy which makes agriculture possible comes of course from an unlimited outside source, the Sun, not taken into account in energy analysis calculations, and there are no figures given for the ways in which different levels of the inputs (fertiliser, agrochemicals a n d mechanisation) affect production.

Figures are given however for the proportions of the agriculture energy budget spent on petroleum, solid fuels, fertiliser and so on which can perhaps give a rough guide to where economies might begin. Petroleum accounts for 34% of the total budget, followed by fertilisers (29%) with machinery and electricity also taking a substantial share.

When figures are given for the ratios of energy (excluding solar energy) input to energy output as metabolisable food energy for various crops and animals they are on the face of it rather alarming even for some of the plants which have the supplementary input of solar energy. But the real question in trying to determine where economies are to be made is to what degree the necessary high levels of production are absolutely dependent on these massive energy inputs. Although the report does discuss this in general terms some facts and figures would have been very useful at this point.

The recommendations in fact concentrate on simple 'waste not' policies; a better use of nitrogen fertiliser, recycling of farm wastes, and some recommendations for research involving a better use of the unlimited energy sources, such as improving the photosynthetic capacity of plants and finding ways of applying biological nitrogen fixation to a wider range of crops. The recommendations often seem not to arise from the energy analysis itself but from the 'old-fashioned' experimental data discussed in the text.   □

Heavenly teacher for Indian pupils

from Narender K. Sehgal, Jullundur

IF all goes well and there are no further postponements, India will in July this year embark on an ambitious experiment in mass education. At the heart of it will be a $205 million American satellite launched in May last year from the Kennedy Space Center in Cape Canaveral, Florida. The most expensive unmanned satellite ever launched by the USA, the Applications Technology Satellite 6 (ATS 6) is designed, among other things, to help

increase literacy in India, and bring televised medical care to Alaska and special educational programmes to rural America.

According to a 1969 Indo-American agreement, the USA will let India use the 1,500 kilogram spacecraft for a year to beam educational television programmes in regional languages to selected villages in the Indian countryside. Called SITE (Satellite Instructional Television Experiment), the project is being coordinated by the All India Radio (AIR) and the Indian Space Research Organisation (ISRO), with assistance from UNESCO, the United Nations Development Programme (UNDP) and the International Telecommunication Union (ITU). Originally planned to start in 1973, SITE got delayed because of two postponements in the satellite launch. The project will now get under way in July 1975 when ATS 6 will be shifted to a position 36,000 kilometres above Kenya. From there, the satellite will receive specially prepared programmes from the Delhi and Ahmedabad stations and relay them to several thousand ground receivers in India. Out of the 6,000 villages in six states—Andhra Pradesh, Bihar, Karnataka, Madhya Pradesh, Orissa and Rajasthan—chosen for the purpose, some 2,400 will employ specially designed 'direct-reception' television sets which will pick up programmes straight from the satellite. Others, within the range of ground transmitters, will use conventional television sets.

An ISRO-built direct-reception television system was recently tested at the NASA Goddard Space Flight Center. During tests, it successfully received signals from the ATS 6. The system transmitted television test patterns from the earth station in Rosman, North Carolina, to the ATS 6.

According to officials of the AIR, by the time the satellite is moved into its appointed position, 35% of the educational programmes intended for use in SITE will have been recorded on video tape. The Earth station at Ahmedabad—modified with assistance from the ITU to transmit as well as receive television signals—will transmit to the ATS 6 these recorded programmes, which will cover education, agriculture, health and family planning. There will also be live transmissions, originating from a television studio at Ahmedabad—built with assistance from the UNESCO—which will include a 10-minute news broadcast each day. The ITU and the UNDP are also providing assistance for the installation of a low power television transmitter, as part of SITE, at Pij, a village near Nadiad in Gujarat. A total of about 1,500 hours of educational programmes will be

SWEDISH students are losing interest in research, especially in mathematics and the natural sciences. The change is part of a general movement away from full-time, examination-oriented university students straight out of school to older, part-time students who have already been employed for a number of years and who take short courses immediately relevant to their jobs and interest. The percentage of part-time students enrolling for the autumn term in Swedish tertiary educational institutions has grown continuously from 12% in 1969 to 29% in 1974.

Students used to compete for a place in a Swedish university. With increasing graduate unemployment, however, the situation began to change, and university applications have been declining since the 1968–69 academic year. This has stimulated the universities themselves to find a new type of student. Members of the new group are, on average, between 27 and 30 years old, have worked for a few years and want to study to enrich interest in the job, without necessarily taking an examination at the end of the course. A typical example is of company employees taking a short course in the economics of the firm. Of all the students who enrolled for this course in the spring term of 1974, only 15% intended to take the examinations.

The trend towards shorter courses has been institutionalised in 'distance teaching': a scheme rather like the UK's Open University except that, instead of centralising the courses under one authority, each of the six Swedish universities runs its own programme. Experiments with these courses have been running on a small scale for a couple of years. But with facilities established for the part-time student without daily access to an educational institution, the trend towards more short courses will probably continue.

All this is obviously beneficial for the new part-time students, but the general trend raises serious questions about the future of academic research. Although short courses in ecology and environmental protection are popular, biology as such has very few takers. There is no lack of applicants for short courses in electronics, energy forms and protection against radiation, but the number of students in mathematics and

Letter from Sweden

from Wendy Barnaby, Stockholm

the natural science faculties has fallen off. Although the surplus of university graduates has been forecast to persist throughout the 1970s, it is predicted that demand from the labour market will catch up with and outgrow supply early in the 1980s. This could have serious consequences not only in the general labour market but also within the universities themselves, in maintaining the number and quality of teaching staff. And it looks as though the country's capacity for scientific research may suffer.

● How would you get rid of your old car if you lived in the countryside far from a scrapping firm, and transportation costs were high? Many Swedes faced with this problem leave cars to rot in country dumps or simply abandon them on by-roads. A recent report commissioned by the Swedish Department of Agriculture suggests measures which could be taken to stop such understandable neglect. According to the report, recirculation of metal and other wastes could save an increasing proportion of the total energy used in Sweden.

The report foresees that recycling could save energy equivalent to 31,000 cubic metres of oil annually, or somewhat less than 1% of the country's total energy requirements. That such a small proportion is seen as justifying a reorganisation of the waste system is indicative of the current energy-consciousness in Sweden. In this affluent, packaged country, where the average household produces 270 kilograms of waste a year a person, scrutiny of energy saving measures is no longer confined to nuclear reactors, wind trappers and sun catchers—it also includes the humble kitchen bin.

The report estimates that more than 20% of household waste could be recycled. At present 70% of the average Swedish kitchen bin is filled with paper, and together these bins produce 780,000 tons of paper a year. The report recommends that householders should be made to separate out the paper from their other garbage, making it easier and cheaper for the municipalities to collect and recycle it. Chemical wastes should be recycled, dumped or deposited on a long term basis by a company jointly owned and run by the government, municipal authorities and industry. And to discourage the dumping of cars, the report proposes a co-operative system of authorisation for scrapping yards and a levy on new cars. The levy, suggested to be Skr.400 (about $US90), would be placed on every new car sold in Sweden after July 1, 1975. When the last owner of the car wanted to dispose of it, he would take it to an authorised scrapping firm (one that met conditions for car disposal, re-sale of spare parts and so on) which would issue a receipt. On presentation of this receipt to the local authorities the owner would be refunded the levy plus a bonus of Skr.100. Since the average life of a Swedish car is 9 years, the system would pay for its own administration as well as providing subsidies to municipal authorities for cleaning up old junk-yards and abandoned cars.

broadcast during the one year experiment. Following the 1969 Indo-US agreement, the SITE Group, specially created in 1970 at Ahmedabad, was charged with the full responsibility for planning, management, operation and evaluation of the experiment. If SITE proves successful, ISRO has its sights set on a multipurpose satellite for providing nationwide television coverage and telecommunication links between Delhi, Bombay, Madras and Calcutta.

In practice, one of the most challenging tasks that SITE is likely to come up against, when the experiment actually begins, will be to keep all the 6,000-odd television receivers (the conventional ones as well as the more expensive direct-reception type) in good working order. This is going to be far more formidable a job than it might appear at first sight. The Haryana government a few years ago installed community television sets in villages within reach of the television station in bordering Delhi. But a check after a short while revealed that most of the receivers had gone wrong because the authorities had not taken steps to ensure proper care, handling, upkeep and maintenance of the sets that were installed. The SITE managers will, one hopes, not allow this to happen.

The SITE project will in many ways be a unique venture. It will, for the first time, demonstrate that relatively inexpensive receivers (the ones built by the ISRO are said to cost about twice as much as the conventional sets) can be used to receive television programmes directly from a satellite. Moreover, such a system could open up immense possibilities and greatly enhance the chances of reducing illiteracy within a relatively short period of time. That is why, ISRO officials believe, SITE results will be of interest to many other countries. □

correspondence

Facts

SIR,—Although I am reluctant to advertise my own philosophical wares in your correspondence column, I believe that some distinctions and definitions made in my book *Scientific Knowledge and Its Social Problems* may be useful for removing some of the confusions referred to in your leader "No longer so much solid ground" (February 13).

There I constructed a sequence of stages of development of materials towards the condition of being "facts" or "knowledge"; and in this it was clear why the published "research report" is not, and cannot be, completely reliable fact. In brief, I start with the problem, and consider the essence of scientific work as the *investigation* of problems (involving creation as well as solution). Contact (necessarily partial and indirect) with the external world is made in the production of *data*, this is then refined to form *information* about the conceptual objects of the scientist's enquiry. But the problem is solved by the *conclusion* to an argument about these objects; and in this structure the information functions as *evidence*. Of course, there is a cyclic interaction between the different phases of this work; and since logical certainty is never possible, it must be governed by socially imposed criteria of *quality* and *value*. For this reason, the social mechanisms of *quality control* are essential for the progress of science; *shoddy* and *vacuous* work (easily explained in this schema) are as "natural" as good work. The *published research report* stating conclusion and evidence is therefore fallible in a number of respects.

The *social* phase of the achievement of scientific knowledge proceeds by judgements of disinterested, competent colleagues. They ask: is this report *relevant* to my enquiry, and if so is it *reliable* for use? If it passes both these tests, it enters the current stock of *tools* for future work. There, it is *transformed* in various ways, depending on the context (descendant problems, application elsewhere, or teaching). If there remains some content that is *invariant* under the inevitable changes in meaning of its terms, then we can call it a *fact*. Facts which survive the demise of the area or field in which they took rise, are then considered as very reliable facts, or bits or *scientific knowledge*.

To be brief, I have omitted all mention of the elaborations of this schema, and of its unsolved philosophical problems. But it seems to provide a reasonable schema of scientific work, and a vocabulary for describing its products.

Yours faithfully,
JEROME R. RAVETZ
London EC4

Probability

SIR,—I wish to suggest the use of a notation which I have found handy, and which seems to fulfil a real, if minor need. It is often necessary to use the phrase "the probability (of some foregoing value or result) under the null hypothesis" or, equivalently, "the probability, given H_0." I recommend that this phrase be rendered as $p \mid H_0$).

Yours faithfully,
M. HAMMERTON
University of Newcastle upon Tyne

Food and allotments

SIR,—Your correspondent F. P. Hughes of Ontario, Canada (February 27) is right in stressing the possible importance of allotments in alleviating a food shortage, but he overstates his case. Allotments in Britain certainly did not produce more than half our food during the world wars. During the 1939–45 war there were 1,500,000 allotments, covering rather less than 150,000 acres of land. There were perhaps as many plots, of a similar area, on which vegetables were grown in private gardens. This area of some 300,000 acres made a useful contribution, perhaps as much as 5%, to our food supplies. It would be difficult to exceed this proportion in most Western countries.

Yours faithfully,
KENNETH MELLANBY
Monks Wood Experimental Station, Abbots Ripton

Teleological vs teleonomic

SIR,—Larkins, MacAuley and MacIntyre (*Nature*, November 29) suggest "a teleological explanation for the siting of the 25 HCC-1-hydroxylase enzyme system in the kidney tubule, where it is able to sense and respond to fluctuations in calcium concentration in the main outflow site from the extracellular compartment".

I take issue with the use of the word teleological in that context. Teleology is defined as the "study of evidences of design in nature". and design as "a

deliberate purposive planning". I doubt that this is the kind of explanation intended by Larkins *et al.* for the siting of an enzyme system in the kidney.

C. S. Pittendrigh in *Behaviour and Evolution*, suggested that the word teleological should be reserved for cases where the idea of the end (goal) precedes the use of the means, and the word teleonomic for cases where the ends result from means that lack design (intent)—as when adaptive traits are produced by random mutations and natural selection.

Accordingly, I suggest that the explanation offered by Larkins *et al.* is teleonomic.

Yours faithfully,
WILLIAM H. GILBERT
*Colby College,
Waterville, Maine 04901*

Taboo research

SIR,—Your correspondent H. Fraenkel-Conrat (March 6) makes one wonder whether after all there are not "two cultures".

Scientists should not think of themselves as the present-day equivalents of Plato's philosopher–kings, who "do good" to the rest of society whether we all will or not.

Robert Boyle wrote that "Gentlemen and scholars are apt to look upon the inquiry into manufactures as a Mechanick employment; and therefore beneath them". We do have, however, HM Factory Inspectorate, with a sound basis of rational "scientific" judgment; and on that basis, we, the whole of society, decide what kind of, for example, dusts, we shall allow in our working lives, and under what conditions and how we shall regulate our exposure to the hazards of our technological culture. By the same reasoning we should surely, the whole of us, decide what it is reasonable to do about self-replicating macromolecules.

Furthermore, Fraenkel-Conrat writes as though there were nothing else to the future of man but his material well-being; surely this is a Procrustean contraction of our attitude to and place in the cosmos—it may well have been for this reason that Prometheus was condemned by the gods to such a cruel punishment as to have his vitals continually eaten by predatory birds and then renewed?

"Man shall not live by bread alone".

Yours faithfully,
R. A. DAVIS
Surbiton, Surrey

news and views

Island biogeography and the design of wildlife preserves

from Robert M. May

ISLANDS have always fascinated ecologists. From the early days of Darwin and Wallace, to the analytical theory of island biogeography developed by MacArthur and Wilson, islands have served as ready made 'evolutionary laboratories' for the development and testing of ideas about the structure of plant and animal communities.

One major aspect of this work seeks to relate the number of species present to the area of the island (which may be a real island in the ocean, or a virtual island such as an isolated hilltop or a wildlife refuge). One rough rule, which fits a surprisingly wide variety of data, is that a tenfold decrease in area corresponds to a halving of the equilibrium number of species present.

In detail, such species–area theories have two components. The first concerns the statics, and seeks to show that there is an equilibrium number of species, S, which is appropriate to an island of given area, A (and that $S \sim A^z$, with z typically in the range 0.2 to 0.3). The second part concerns the dynamics, the way the equilibrium is maintained by a balance between extinction and immigration. One line of evidence comes from studying the increase towards equilibrium on islands where the flora and fauna have been removed either by natural catastrophe (as in the case of Krakatoa in 1883), or by experimental manipulation (as in the removal of the arthropod fauna from tiny, four square metre, islets off Florida by Simberloff and Wilson). Similarly it is revealing to monitor the initial colonisation of a newly created volcanic island such as Surtsey. A second line of attack is to study the way the balance is maintained on islands which are thought to have settled into their equilibrium state (for example, some of Lack's studies, or Diamond's study of the California islands); such work is frequently beset by problems in estimating man's effects on natural turnover rates. A third approach focuses on offshore 'land bridge' islands, which in the heyday of the last ice age some 10,000 years ago

(when the oceans were typically 100 metres lower) were part of the mainland area; as these islands have been created by the rising oceans, their rich complement of mainland species has slowly declined towards the island equilibrium value. Studies by Diamond on land bridge islands in the vicinity of New Guinea, and by Terborgh (chapter 24 in *Tropical Ecological Systems*, Ecological Studies Vol. 11; Springer-Verlag, 1975) on neotropical land bridge islands such as Trinidad and Tobago, show a very consistent pattern in their rates of relaxation towards equilibrium in the species number.

Recently several people have observed that these notions have relevance to the design of national parks and wildlife preserves.

At a qualitative level, it follows that the number of species naturally maintained at equilibrium depends on the size of the park. Many species will not cross major highways (for good reasons!), and for these species the effective area of the park is halved by bisecting it with a big road: about one sixth of all such species will be gone when the system settles to its new equilibrium. In cases where one large area is infeasible, it must be realised that several smaller areas, adding up to the same total as the single large area, are not biogeographically equivalent to it: they will tend to support a smaller species total. One way to raise the equilibrium number of species in any one such smaller park is to raise the immigration rate into it. This can be done by judicious juxtaposition of the scattered parks, and by providing corridors or stepping stones of natural habitat between them. These and other aspects of the "geometry of wildlife refuges" have been developed by Jared Diamond in an invited address at the 16th International Ornithological Congress in Canberra, 1974 (proceedings to be published), and by E. O. Wilson and E. O. Willis (in a chapter of *Ecology of Species and Communities*, to be published by Harvard University Press, 1975).

These articles represent but the beg-

innings of the subject. They ignore, among other things, epidemiological aspects of park management: many scattered parks have over a single park the advantage that not all eggs are in the one basket. Moreover species–area relations only aim at a coarse-grained first approximation to the floral and faunal dynamics. They do not incorporate such things as the spatial and temporal patterns of stable oscillation which so frequently characterise populations both in mathematical models and in the real world. The botanical evidence for a 50 year cycle in elephant irruptions in the area that is today Tsavo National Park at the foot of Mount Kilimanjaro (for example, G. Caughley, *E. Afr. Wildl. J.*, in the press) suggests that such dynamic features of natural populations can create management problems when even a very large area is enclosed.

One example of a quantitative application of these ideas is given by M. B. Usher's study of the relation between the areas of twelve nature reserves in Yorkshire and the number of species of higher plants growing in them (in *Biological Management and Conservation*; Chapman and Hall, 1973). The observations fit the relation $S \sim A^{0.21}$, with the park areas varying over more than two orders of magnitude.

A particularly nice calculation has been made by John Terborgh, in the article referred to above. For the West Indian land bridge islands, he knows the number of bird species currently to be found on a given island, and can estimate (from the neighbouring mainland species abundance) the number which were present before the island was cut off 10,000 years ago. This gradual decline in species number on the islands may be described by an equation

$$dS/dt = kS^2 \qquad (1)$$

Here k is a measure of extinction rate, and Terborgh takes the overall loss rate to have an interactive form, proportional to S^2, to account for the fact that species hasten each other's extinction. A more detailed empirical and

theoretical justification for the use of this equation was independently given by T. W. Schoener at the 16th International Ornithological Congress, mentioned above. The extinction rate k may now be calculated for each island. As expected, the k values have a systematic dependence on island area.

Now comes the payoff. The island of Barro Colorado was created with the formation of Lake Gatun in conjunction with the construction of the Panama Canal, and since 1923 it has been carefully protected as a wildlife preserve. A meticulous account of the bird species present on the island over the past 50 years has recently been published by E. O. Willis (*Ecol. Monogr.*, **44**, 153–169; 1974). From the k-versus-area patterns of West Indian land bridge islands, an estimate of the k value for Barro Colorado island may be obtained. Equation (1) can then be used to get an independent estimate of the decline in the island's number of bird species since 1923. Terborgh's theoretical estimate of 16–17 species lost is reassuringly in accord with the actual number observed by Willis, namely 15.

Proteins which bind polyenes and react with photons

from A. J. Thomson

UNTIL a decade ago rhodopsins were the only proteins known to employ a retinyl polyene as a prosthetic chromophore. A large number of rhodopsins have been found (see Knowles, *Nature*, **253**, 394; 1975). All have the common feature of the 11-*cis* conformation of the polyene chain which in the examples investigated is bound to the protein through a protonated Schiff base linkage. The action of light is to produce the all-*trans* isomer with concomitant loss of a proton.

Three additional types of protein–polyene complexes have been discovered within the last few years, revealing an intriguing variety of functions and a satisfying economy of use of prosthetic group. The newcomers, in order of appearance, are first the yellow vitamin A transporting complex, called retinol binding protein (RBP), characterised by Kanai, Raz and Goodman (*J. clin. Invest.*, **47**, 2025; 1968). This protein stabilises and solubilises in water all the mono-*cis* and the *trans* isomers of vitamin A. No photochemical reactions of this complex have been reported. The second, retinochrome, was found by Hara and Hara (*Nature*, **206**, 1331; 1965) in various kinds of cephalopod retina, such as that of the Japanese squid. This protein absorbs maximally in the range 490 to 522 nm and contains the all-*trans* isomer of the retinyl polyene bound to the protein by a Schiff base link, probably protonated. The action of light is to release the 11-*cis* isomer of retinol. The most surprising of the newcomers, however, is bacteriorhodopsin, a purple pigment found in the outer membrane of the bacterium, *Halobacterium halobium*, when it is grown in low oxygen tension (Oesterhelt and Stoeckenius, *Nature new Biol.*, **233**, 149; 1971). Containing the 13-*cis* isomer of the retinyl polyene chain linked to the protein also by means of a protonated Schiff base, the action of light is to produce the all-*trans* isomer with the loss of a proton. The polyene chain is not detached from the protein but returns in a dark, thermal step to the protonated 13-*cis* form (Oesterhelt, Meentzen, and Schumann, *Eur. J. Biochem.*, **40**, 453; 1973; Oesterhelt and Stoeckenius, *Proc. natn. Acad. Sci.*, *U.S.A.*, **70**, 2853; 1973). The result of this rapid light-driven cycle is the transport of protons across the membrane and the accompanying production of ATP. This protein is now an important tool in the study of energy coupling driven by electrochemical gradients of the type first suggested by R. J. P. Williams (*J. Theoret. Biol.*, **1**, 1; 1961) and P. Mitchell (*Nature*, **191**, 144; 1961).

The three proteins retinochrome, bacteriorhodopsin, and rhodopsin provide an opportunity for comparative studies of the part played by a protein in directing the course of a photochemical reaction. The three light-induced reactions observed are all-*trans* → 11-*cis*, 13-*cis* → all-*trans* and 11-*cis* → all-*trans*, respectively, the last two also involving loss of a proton at some stage. Retinochrome is unique in being able to catalyse, in the presence of light, the isomerisation to the 11-*cis* form of the 9-*cis* and 13-*cis* isomers of retinol as well as the all-*trans*. The quantum efficiency of the reaction is not known in the case of retinochrome but for rhodopsin and bacteriorhodopsin the efficiencies are remarkably high, 0.65–0.7 and 0.79, respectively. This poses the interesting question of how the protein can direct a photochemical reaction so efficiently along various reaction coordinates or, indeed, in opposite directions along the same coordinate.

Catalysis of an excited state reaction must overcome the fact that non-radiative decay by vibrational relaxation to the ground state can be very fast, $\sim 10^{11}$–10^{12} s^{-1}. In order to become efficient the required photoreaction must be faster than these competing processes. It was therefore not wholly surprising that measurements of the rise time of the earliest detectable intermediate at 560 nm after photolysis of bovine rhodopsin gave a value of less than 6 ps, the experimental resolution. This was taken as evidence that the intermediate, pre-lumirhodopsin, is the first product of the primary photoprocess (Busch, Applebury, Lamola and Rentzepis, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2802; 1972). It was however recognised by these authors that the nature of a primary photoprocess occurring within $\sim 10^{-12}$ s is severely constrained. Complete *cis–trans* isomerisation is unlikely to take place within this time. These workers suggested on the basis of a recent crystal structure analysis of 11-*cis* retinal (Gilardi, Karle and Karle, *Acta Cryst.*, **B28**, 2605; 1972) that the conformational change involves only a rotation of the central portion of the chain from the 11-*cis*, 12-s-*cis* form to 11-*trans*, 12-s-*cis*.

If it is generally true that unimolecular photoreactions must have a very fast primary step in order to reach high quantum efficiencies, then it is interesting to ask what are the likely primary photoreactions of other polyene–protein complexes. The overall reaction of bacteriorhodopsin involves an isomerisation step and proton loss, as does that of rhodopsin. Which process is the primary fast step? In both proteins a number of intermediates have been identified by their spectral properties either by freezing out at low temperatures or by use of fast detection techniques (Lozier and Stoeckenius, *Fedn Proc.*, **33**, 1408; 1974: Slifkin and Caplan, *Nature*, **253**, 56; 1975). The technique used by Slifkin and Caplan is novel, involving the excitation of bacteriorhodopsin membrane with a modulated light source followed by phase-sensitive detection, at the modulation frequency, of changes in the optical absorption of a monitoring beam. No doubt this is a difficult experiment to perform especially with a scattering material. Resonance Raman spectroscopy has provided the first direct observation of the protonated Schiff base linkage and shows that, in bacteriorhodopsin, the native protein contains this grouping whereas the proton is not present in the link after irradiation with light to produce a species absorbing at 412 nm (Lewis, Spoonhower, Bogomolni, Lozier and Stoeckenius, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4462; 1974). This result corrects an earlier conclusion, also based on Raman data (Mendelsohn, *Nature*, **243**, 22; 1973). The same technique has also been used to show that an early intermediate of rhodopsin, pre-lumirhodopsin, is

protonated (Oseroff and Callendar, *Biochemistry*, **13**, 4243; 1974).

The difficult question to resolve is which species are primary photoproducts leading to a biochemical event and which competing by-products. Since the quantum efficiencies of formation of these species are not known and since the quantum efficiencies of the photobiological processes of rhodopsin and bacteriorhodopsin are less than unity, this question remains open. In view of the function of bacteriorhodopsin it is worth examining the hypothesis that the loss of a proton from the excited Schiff base may be the primary photoprocess. Involving the motion of a proton along the N–H vibrational coordinate this transfer will be exceedingly fast, within the time of a single vibration. All that is required is a change in the basicity of the N atom in the excited state. If this were followed by a slower thermal isomerism of the polyene chain the role of the protein in selecting the course of the isomerism becomes more comprehensible. One possible test of this hypothesis might be made by measuring the quantum efficiencies of the photoprocesses of rhodopsin and bacteriorhodopsin after deuteration of the Schiff base link. A pronounced isotope effect should be seen only if proton transfer is involved in the primary photoprocess.

Globin synthesis: rates and ratios

from Pamela Hamlyn

It has been known for several years that the α and β globin chains of mammalian haemoglobin are synthesised on different polysomes and yet their production is controlled in such a way that nearly equal amounts of each are produced. The means by which these two independent events are synchronised is the subject of some interest for it seems possible that this mechanism may be one of general importance in the control of synthesis of proteins composed of two or more subunits.

Lodish and his co-workers have made several contributions to the study of globin synthesis control and recently Lodish (*Nature*, **251**, 385; 1975) has derived a simple kinetic rate equation for initiation and elongation of polypeptide chains which, although based on α and β globin synthesis, may be applicable to eukaryotes in general. A result of these calculations is the prediction that any reduction in the rate of polypeptide chain initiation will lead to preferential inhibition of translation of mRNAs with lower rate

constants for polypeptide initiation. Lodish has argued that α mRNA has a lower initiation constant than β mRNA. Based on the observation that α mRNA is found in polysomes averaging three ribosomes, whereas β mRNA polysomes have five ribosomes on average, he contends that since the rate of elongation is the same for α and β chains then α mRNA must initiate protein synthesis only 60% as often as β mRNA. (He suggests that balanced synthesis of the two globins is achieved by a 1.7-fold excess of α mRNA over β mRNA.) Globin synthesis, therefore, provides an experimental situation to test the predictions of his model. Accordingly Lodish has added inhibitors of protein synthesis initiation to a reticulocyte lysate and measured the effect on the ratio of α and β globin synthesised. In all cases he found that it was α mRNA translation that was preferentially inhibited as predicted by his model.

McKeehan, working at the Basel Institute for Immunology, has reported the results of a systematic study of globin synthesis in the reticulocyte lysate in which each of the components of the synthesis was varied in turn—keeping the others constant—and the effect on the α/β globin ratio measured (*J. biol. Chem.*, **249**, 6517; 1974). He found that increasing the concentration of globin mRNA resulted in a decrease in the α/β globin ratio, whereas increasing the concentration of 'initiation factors' (obtained from a salt wash of the small ribosomal subunit), or the concentration of the ribosomal subunits, resulted in an increase in the α/β globin ratio. When experiments were performed with separated subunits it was found that increasing the 40S subunit relative to the 60S gave an increase in the α/β globin ratio, whereas when the 60S subunit was in excess then there was a small decrease in the α/β globin ratio.

McKeehan maintains that these results can best be explained using Lodish's assumptions, namely that β globin mRNA initiates the synthesis of protein more efficiently than α mRNA, and also that there is more active α mRNA than β mRNA bound to polysomes.

Consider for example when the concentration of 40S ribosomal subunit is increased, all other components being held constant. The excess of small subunit means that α and β mRNA are no longer in competition for initiation so that the superior efficiency of β mRNA initiation is not involved in determining the α/β globin ratio produced in the reticulocyte lysate. Only the relative concentrations of the two messengers is effective in determining the globin ratio and since there is more α mRNA then the α/β globin ratio

increases, as is observed experimentally.

Both Lodish and McKeehan make the point that these mechanisms for altering the ratio of α/β globin synthesised rely only on varying the relative amounts of the components of protein synthesis and do not require existence of α and β mRNA specific initiation factors.

The results and predictions of Lodish and McKeehan go some way to defining the components in the mechanism of control of globin synthesis but do not explain how the feedback could operate *in vivo*. In other words, adding more 9S RNA to the constituents of protein synthesis may increase the relative amount of β globin but how is the lack of β globin communicated so that more 9S mRNA is made available to the ribosomes?

rosy structural gene for xanthine dehydrogenase

from Benjamin Lewin

WHAT is a gene? This question has been resurrected by the present debate on the nature of the unit of gene expression in eukaryotes. At the heart of the discussion is the apparent discrepancy between the coding potential of the eukaryotic genome and its probable number of genes. This can be placed on a satisfactory quantitative basis really only with *Drosophila*, where a visible division of the genetic material into about 5,000 bands is evident in the polytene chromosomes of the salivary glands. That each band represents a single genetic function is suggested by the identification of individual lethal complementation groups with single bands in a region of the X chromosome (Judd, Shen and Kaufman, *Genetics*, **71**, 139–156; 1972) and by the rough equality between the total number of sex-linked lethals and X chromosome bands (Hochman, *Cold Spring Harb. Symp. quant. Biol.*, **38**, 581–598; 1973) and between the total number of third chromosome late lethals and bands (Shearn, *Genetics*, **77**, 115–126; 1974). But the average content of a band is about 30,000 base pairs, sufficient to code for 10–20 proteins of average size. Two extreme views can be taken of this situation: all genes may be identifiable by lethal mutations, so that each band contains only one protein-coding sequence, on average occupying about 10% of the DNA; or only a small minority of genes can be identified by lethal mutation, so that there may be many 'null loci' that are unidentifiable by mutation, in which case there might

be some 50,000 genes with many in each band (see Lewin, *Nature,* **251,** 373–375; 1974).

Another approach to defining the genetic unit is to determine the nature of mutations at some locus. The mutations mapping in one complementation group may include *cis*-dominant control elements as well as structural genes; since most loci are identified by the effects which mutations in them have upon the phenotype, there

is usually no way to tell whether they represent alterations in a control element or structural gene. To determine the class of mutational event therefore demands analysis of loci where a phenotypic effect can be recognised and the protein product is known.

rosy mutants in *Drosophila* have eyes that are brownish in colour (instead of the wild type red colour). Two sets of observations suggest that this locus is connected with the enzyme xanthine

dehydrogenase. A dosage effect exists in which heterozygotes with only one ry^+ gene possess half of the wild type level of enzyme activity; and abnormal flies with three ry^+ genes have 150% of the wild type level (Grell, *Z. Vererb.,* **93,** 371–377; 1962). This could mean that *rosy* mutations act in a control element to prevent synthesis of XDH or that they represent alterations in the structural gene completely preventing enzyme activity. Isoalleles that affect the electrophoretic mobility of XDH (but which are wild type in eye colour) map close to the *rosy* mutations, an indication that the structural gene is in or close to the *rosy* locus (Yen and Glassman, *Genetics,* **52,** 977–981; 1965). By fine mapping of some of these isoalleles, Gelbert, McCarron, Pandey and Chovnick (*Genetics,* **78,** 869–886; 1974) now define their relationship to *rosy* mutations in a report on the organisation of this locus.

rosy mutants entirely lack XDH activity, since even low levels of this enzyme are sufficient to allow expression of the ry^+ phenotype, so that it is the ry^+ isoalleles alone that can be used to define the structural gene by their effect on the mobility of the enzyme. Since it is not practical to map such sites directly, the first step in this analysis was to derive *rosy* mutants from each of five of the ry^+ isoalleles. When two such *rosy* mutants are crossed, ry^+ progeny can be selected by growth on a purine-supplemented medium which kills larvae lacking XDH. In these experiments, 170 ry^+ survivors were obtained from a total of 7×10^6 zygotes. The markers present on either side of the *rosy* locus were used to determine whether a crossover or gene conversion event was responsible for generating the ry^+ progeny. Examination of these strains to determine whether they have the XDH electrophoretic mobility characteristic of the maternal or paternal parent can then be used to map the site responsible for the electrophoretic variation. In effect, the *ry* mutant sites are selected markers in these crosses and the sites responsible for electrophoretic mobility comprise unselected markers.

The electrophoretic (*e*) sites mapped in two clusters, one at each end of the region identified by *rosy* mutations. This leaves open the possibility that the structural information for XDH might be located in two elements separated by some distance. By using a *cis/trans* test, however, Gelbart *et al.* demonstrated that *e* sites at both extremes appear to fall within the same protein-coding element. This is consistent with the observation that the enzyme XDH consists of a dimer of two identical subunits of about 130,000 daltons; this should require a coding length of a little more than 3,000 base

Strong interactions of the psi

from David J. Miller

SINCE the ψ (or J) particles were first discovered at Brookhaven and the Stanford Linear Accelerator Centre in November 1974 (see *Nature,* **252,** 438; 1974 and *Phys. Rev. Lett.,* **33,** 1404, 1406 and 1408; 1974), experimental physicists all over the world have been looking for their effects. Some have scanned the data already collected from previous experiments, others have made proposals for new and more refined experiments, but much of the data now appearing is coming from apparatus which just happens to be set up at the accelerators or storage rings, and which has been rapidly adapted to look for the new particles.

The world of physics has been swept by waves of gossip, often inaccurate, and it has become necessary to separate 'mezzofacts' from real facts which can be reliably traced back to their authors.

The latest pieces of information to leave the mezzo-fact category are data on the photoproduction of the ψ (or J) with a mass of 3.1 GeV/c^2. The Brookhaven experiment showed that this ψ is produced with a very low probability in proton–proton collisions. The SLAC, and other subsequent e^+e^- experiments, saw copious production. These two results implied that the ψ takes part in electromagnetic interactions, but might not have strong interactions with nucleons (protons or neutrons). In photoproduction, a particle is produced by bombarding a nuclear target with high-energy γ rays. If the ψ is a 'vector' particle, like the φ meson (as discussed in *Nature,* **252,** 438; 1974), then an interacting photon could sometimes behave as if it were an incoming ψ. The photoproduction of the φ, and the related ρ and ω vector mesons, are very well explained by treating the incoming photon as a vector meson, which then has a strong interaction with the target nucleon. This is called the 'vector meson dominance model'

(VMD) of photon–hadron couplings. If the ψ is a hadron (a strongly interacting particle) then the model would predict the rate of ψ photoproduction, with only two important parameters: the strength of the coupling of γ to ψ, which is measured directly by the $e^+e^- \to \psi$ experiments, and the cross section (a direct measure of probability) for ψ–nucleon scattering.

Three groups have produced results in searches for ψ photoproduction. At the Cornell electron synchrotron (*Phys. Rev. Lett.,* **34,** 231; 1975) no sign of ψ production has been seen with 11 GeV photons. An experiment at the SLAC linear accelerator has also seen no ψ production from 18 GeV photons (*Phys. Rev. Lett.,* **34,** 288; 1975). But news has come from the Fermi National Accelerator Laboratory near Chicago that a group led by Professor Wonyong Lee of Columbia University has seen ψ photoproduction by photons of about 100 GeV produced from the 400 GeV proton-synchrotron. This result was a mezzo-fact for some months, but has now been reported by Lee himself in an FNAL seminar and can be regarded as authentic.

The tentative rate for ψ photoproduction at FNAL appears consistent with the limits put upon it by the two experiments which saw no effect. Using the VMD model it is clear that Lee's result implies a cross section for ψ–nucleon collisions somewhere in the region of one millibarn (10^{-26} cm^2). Only the strong interaction could give such a large cross section, but the cross sections of other strongly interacting particles are all in the region of 20 to 40 millibarns. The ψ therefore seems to be a 'semi-strong' particle. It is likely that this inhibited interaction probability is linked to the narrow width of the ψ and slowness of its decays, but there is no agreement on what the link may be.

airs. This length fits well with the enetic analysis of *rosy*, since *ry* iutants span 9×10^{-3} recombination nits, and 0.01 units has been estimated o represent almost 4×10^3 base pairs. he *rosy* locus lies in a region of five ands on the polytene third chromo- ome and since these are of average ze this implies that whichever band ontains *rosy* has about ten times more)NA than codes for the protein. The *osy* site, lying at map position 52.0, is ounded by *kar* at position 51.7 on one ide and by *l(3)26* at position 52.2 on he other and the isolation of further iutations may, of course, permit a iore exact resolution of its extent.

If it is true that the distribution of iutant sites within control elements nd structural genes directly reflects ieir relative sizes, these results show hat the *rosy* locus represents a struc- ural gene; for if only part of the egion identified by *rosy* mutations had he function of coding for protein, the ites responsible for electrophoretic ariation would lie in one small cluster nstead of extending to both ends of he genetic map. In support of this ontention, Gelbart *et al.* argue that he *rosy* mutations which they selected y X-ray mutagenesis largely represent ingle base pair alterations. Point nutations in this case must be able to bolish completely the activity of XDH. ly using mutagens inducing other types f changes that perhaps might be prone o have effects on control elements, in he future it may be possible to identify egulatory *rosy* mutations. The conclu- ions that the *rosy* locus is largely iccupied by the structural gene for <DH depends also upon the assumption hat recombination rates are constant ier unit length of DNA throughout ioth control element and structural ;ene; for if recombination takes place it a greater frequency in the structural ;ene than in the control element, the ipparent size of the structural gene vould be exaggerated relative to the iontrol element. The failure of Chov- iick and his colleagues to observe inequal crossing over in their earlier xtensive analysis of recombination at he *rosy* locus argues that the structural ;ene itself comprises nonrepetitive)NA, but leaves open the possibility now under study) that adjacent control iements might represent repetitive equences in which recombination is iuppressed in order to limit such events. he most probable explanation of these esults therefore seems that the *rosy* icus represents the structural gene for <DH, occupying only some 10% of the and in which it is located. Whether the emaining DNA represents control inctions associated with the XDH iructural gene and thus defines a single enetic unit remains a point for future esearch.
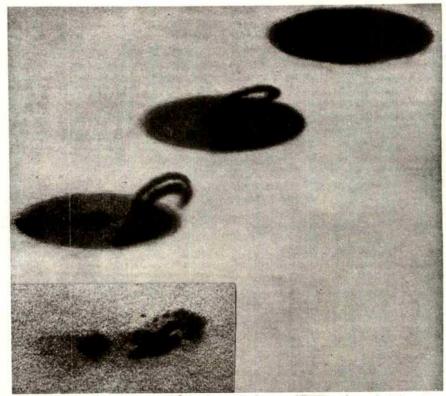
Ring galaxies and intergalactic gas clouds

from Craig D. Mackay

IN a recent article in *The Astrophy- sical Journal* (**194**, 569; 1974), Freeman and de Vaucouleurs attempt to account for the formation of two types of object usually dismissed as "irregular galaxies". The first appears as an ir- regular ring of gas with a number of condensations within it, the second (an example is shown at the bottom of the accompanying figure) appears as a spheroidal galaxy composed almost ex- clusively of stars with a small region filled with several blobs of gas.

The ring galaxies have a number of features similar to those of the annulus of gas found in many spiral galaxies. Freeman and de Vaucouleurs con- sider the possibility that such an annulus was stripped out of a normal spiral galaxy to form a ring galaxy by a collision with an intergalactic gas cloud. The presence of large clouds of neutral or ionised gas moving within clusters of galaxies has been suggested as a convenient way of stabilising clusters of galaxies. The scenario proposed is as follows: Suppose that a gas cloud collides with a normal spiral galaxy as is shown in the artist's impression in the upper part of the figure. Stars, being small and massive, are not affected by the colliding gas cloud. The annulus of gas, however, is

progressively stripped out of the galaxy as shown in the figure. Some of this gas may be attracted back towards the massive disk of the galaxy, giving rise to the 'hook' in the lowest of the artist's sketches, a sketch which looks remarkably like the object NGC 7828–29 shown at the bottom of the figure. Eventually all the gas is swept out of the galaxy and a spinning ring of gas is formed moving away from the parent galaxy. This gas ring is unstable and expands (because of the unbalanced centrifugal force) until it is braked by the background gas in the cluster. On fairly short time scales ($\sim 10^8$ years) in- stabilities in the gas cause knots to condense in the ring giving structures very similar to the ring galaxies ob- served.

The main difficulty with Freeman's and de Vaucouleur's model is that it is important the collision happens rapidly enough to ensure that all the gas is swept out in a small fraction of the rotation time of the galaxy (otherwise the ring structure will become grossly distorted). A second problem is that it is difficult to avoid heating the gas ring in such a collision to an extent that the ring structure is completely disrupted. There is, however, no doubt that their model gives a compelling account of the formation of these hitherto un- explained galaxies. Indeed, the elegance of their model ensures that it will receive further serious attention and may help to explain a number of the many other unusual galaxies which are all around us.

Artist's conception (above) of three typical phases of penetration of disk galaxy passing through an intergalactic cloud, with progressive separation of gas annulus from disk; (bottom) photograph of NGC7828–29.

Seismic activity before earthquakes

from Peter J. Smith

IT is now well known that many moderate and large earthquakes are immediately preceded by smaller foreshocks; that is, over periods ranging from weeks to hours before significant seismic events there is an increase in seismic activity. But what happens to the seismicity in the vicinity of an earthquake epicentre during the preceding months and years? Here opinions are more divided, with some studies concluding that the level of regional seismicity also generally increases over the longer time scale and others suggesting that earthquakes are frequently heralded by a period of relative quiescence.

Mogi (*Bull. Earthquake Res. Inst.*, **47**, 395; 1969), for example, concluded from a study of four great Japanese earthquakes that whereas the immediate epicentral area becomes relatively calm for some years prior to the main shock, activity in the surrounding regions increases markedly. He likened the pattern of seismicity to the shape of a ring doughnut centred on the epicentre. A somewhat similar pattern was also observed by Borovik *et al.* (*Izv. Acad. Sci. USSR, Phys. Solid Earth*, **2**, 21; 1971) in a quite different tectonic setting. Borovik and his colleagues found that the area surrounding the 1959 Baykal (Soviet Union) earthquake was relatively quiet seismically for several years before the event and that the quiet area, or 'preparation region', enclosed the aftershock zone. During the 7½ years preceding the main earthquake, only two (smaller) earthquakes were observed within the preparation region whereas 20 were recorded in an outer region of comparable area. After examining the geological structure and seismicity of southern California, Allen *et al.* (*Bull. Seismol. Soc. Amer.*, **55**, 753; 1965) even went so far as to propose that areas of seismic quiescence may indicate the sites of future large earthquakes.

Fedotov *et al.* (in *Earthquakes and the Deep Structure of the South Kurile Island Arc*, Moscow, 1969) have taken precisely the opposite view, arguing from their study of the Kamchatka–Kurile–Japan seismic zones that impending great earthquakes are heralded by an increase in seismic activity beginning 5–20 years before. In this they are supported by Sadovsky *et al.* (*Tectonophysics*, **14**, 295; 1972) who suggested on the basis of Asian studies that an increase in seismic activity over a 5–10 year period indicates a forthcoming strong earthquake. Suyehiro and Sekiya (*Tectonophysics*, **14**, 219;

1972) also found that the seismicity in the vicinity of the great Kanto earthquake of 1923 was significantly greater during the few years before the shock than over the period 1926–1972. And finally, Tocher (in *San Francisco Earthquakes of March 1957*, California Division of Mines and Geology, Spec. Rep. 57, 1959) concluded that for several decades preceding the Hayward earthquake of 1868 and the San Francisco earthquake of 1906 moderate earthquakes probably occurred at a greater frequency than they do on average over the longer term. He thus interpreted increasing regional seismicity as a symptom of a strain buildup which could subsequently lead to a major shock.

So which side is right? Or is the conflict more apparent than real, either because different earthquakes are associated with genuinely different precursory phenomena or because experimental and analytical techniques have hitherto been imperfect? In an attempt to resolve the matter, Kelleher and Savino (*J. Geophys. Res.*, **80**, 260; 1975) have now carried out an extensive study of the patterns of seismic activity preceding large strike-slip and thrust-type earthquakes which have occurred along the northern, northwestern and eastern margins of the Pacific. The events include the San Francisco earthquake of 1906, the Kamchatka event of 1952, the Chile shock of 1960, the great Alaska earthquake of 1964 and several others, most of which had rupture zones extending hundreds of kilometres. In each case the foci of all shallow (<100 km) events preceding the main shocks were relocated, in some cases for precursory intervals of up to 45 years.

The observed patterns of seismicity preceding large earthquakes were found to correlate with the configurations of the shocks' rupture zones and with the positions of the main event epicentres within the zones. Specifically, extensive parts of the rupture zones were relatively aseismic until the times of the main shocks, and the low levels of seismicity applied to events at least several magnitudes smaller than the main shocks and probably to events many magnitudes smaller. On the face of it, this would seem to support those arguing for precursory quiescence; in practice, the detailed picture looks rather different. In most of the earthquakes examined by Kelleher and Savino rupture began in an area of moderate seismic activity, propagated up to hundreds of kilometres into adjacent quiet regions, and in many cases ended in areas with marked prior seismicity. Thus prior seismic activity often occurred near the epicentres of the main events and near the edges of the rupture zones but not throughout

the main bodies of the zones.

These general principles are w illustrated by the Kamchatka eart quake of 6 November 1952. During t earthquake, rupture propagated fro the focus towards the south-we parallel to the strike of the thrust pla and throughout an elongated zone 40 500 km long and 100–200 km wi For 30 years before the earthqual most of the rupture zone was relative quiet seismically; but throughout t period there was a clustering of activ around the position of the subseque epicentre at the north-east of the ru ture zone and around the oppos (south-western) end of the zone. T bulk of the rupture zone only becar seismically active with the aftershoc of the main earthquake.

There is some indication from t Kamchatka and other large eart quakes that, where prior activity dc occur (especially in the epicentr regions), its level increases as the ma shock approaches. On the one han there is no suggestion that precurso seismic activity appears in the ga where none occurred before. Gaps seismicity for great earthquakes alor major plate boundaries seem to gaps for smaller magnitude activi also; and these gaps remain until a unless they become aftershock zone Unfortunately, the available data a insufficient to enable temporal var tions to be determined in any gre detail. Thus it is not yet possible to sa whether a seismic gap becomes quie cent as part of a definite process leadi to a large earthquake or whether, on the aftershocks of a large event ha died away, the gap remains quiesce until the next large shock. At prese there are data to support either b haviour but not enough to deci which predominates. What the ne study by Kelleher and Savino has mac clear, however, is that the spatial di tribution of seismicity is critical in an attempt to describe temporal vari tions. It is not enough to say that particular earthquake was preceded t relative quiescence or a regional i crease in the level of seismic activit The precise points at which thes phenomena do or do not take plac must be clearly determined.

Turnover of motor endplate

from Angela Vincent

THE transmission of impulses at th neuromuscular junction is achieved b the release of acetylcholine from th nerve ending. Acetylcholine interac with receptors on the endplate of th muscle and causes a change in sodiu and potassium permeability whic

results in propagation of the impulse along the muscle fibres and activation of the contractile apparatus In mammalian skeletal muscle the endplate has a diameter of 20–60 μm, but its surface area is increased by infolding of the postsynaptic membrane The acetylcholine receptor in normal muscle is confined almost exclusively to the endplate, as has been shown autoradiographically by labelling with the specific and irreversible blocking agent α-bungarotoxin (α-Bgt) (Lee, Tseng and Chiu, *Nature*, **215**, 1177, 1967, Barnard *t al*, *Nature*, **234**, 207, 1971) Recent estimates of receptor sites suggest a density of $2–4 \times 10^4 \mu m^{-2}$ (Fertuck and Salpeter, *Proc natn Acad Sci U S A*, **1**, 1376, 1974, Porter and Barnard, *J Membrane Biol*, **20**, 31, 1975) When the nerve to a muscle is cut, acetylcholine sensitivity begins to appear over the entire surface of the muscle fibre and reaches a maximum in 2–3 weeks, only declining as reinnervation takes place (Axelsson and Thesleff, *J Physiol*, *Lond*, **147**, 178, 1959, Miledi, *Physiol*, *Lond*, **151**, 241, 1960) Radioactively labelled α-Bgt binding gain follows this closely binding increases 20-fold overall (Miledi and Potter, *Nature*, **233**, 599, 1971), but the density of extrajunctional sites, at peak 600 μm^{-2} (Fambrough, *J gen Physiol*, **4**, 468, 1974), never approaches that at the endplate itself Despite the translation freedom found for many membrane-bound proteins the normal endplate receptors remain discretely localised—even after denervation and degeneration of the nerve ending

What makes the endplate so stable? Berg and Hall (*Science*, **184**, 473, 1974) labelled rat denervated diaphragm with ^{125}I-α-Bgt *in vivo*, removed the muscles and cultured them for 24 hours They found that the extrajunctional radioactivity declined with a half life of 8 h, whereas there was only a marginal reduction in endplate labelling during that time The rate of ^{125}I loss was reduced considerably by metabolic or protein synthesis inhibitors, indicating that little of it was due to reversibility of the toxin–receptor complex Radioactivity recovered from the medium as mostly degraded toxin, although native toxin added to the culture was not broken down They concluded that the extrajunctional toxin–receptor complex was being metabolised considerably faster than the endplate complex Chang and Huang (*Nature*, **253**, 43, 1975) confirmed these findings, but they left the ^{3}H-α-Bgt-labelled diaphragms *in situ* for several days before removing them They found that the turnover of endplate receptors had a half life of about 7 5 days which could be shortened by conditions which are known to increase the synthesis of new receptor, such as denervation per-

formed at the same time as labelling Their value for the half life of extrajunctional receptors in rats denervated 9 days previously was 19 hours, somewhat longer than Berg and Hall found in rats which had been denervated 5 days previously

Does this different turnover reflect differences in the receptor molecules themselves, or in their mode of incorporation into the membrane? Several reports suggest that the two classes of receptor in intact muscle are not identical—with respect to their sensitivity to cholinergic antagonists (Beranek and Vyskocil, *J Physiol*, *Lond*, **188**, 53, 1967 and Lapa *et al*, *Exp Neurol*, **43**, 375, 1974) and their acetyl-

A hundred years ago

The third paper was by Mr B Tower, on a method of obtaining motive power from wave motion He said that this inquiry originated with Mr Deverell, who came home from the antipodes for the purpose of promulgating it Mr Deverell's proposition was to suspend a heavy weight on board a ship by means of springs, and to obtain motive power by the oscillation of this weight through a distance not more than the height of the waves It however appeared to Mr Tower that since the centrifugal force of wave motion in a vertical direction is alternately added to and subtracted from, the force of gravity thereby causing a virtual variation of the intensity of that force, the question might be broadly stated as follows —

Supposing the force of gravity to vary in intensity at regular intervals, that is, to become alternately greater and less than its normal amount, what is the best means to obtain the maximum amount of energy from a given weight oscillating under the influence of these variations?

Now as energy or power is defined as force moving through distance, it is clear that the quantity of energy or power to be obtained by this system will depend on the distance through which this weight is caused to move during each successive variation of gravity

The first experiments Mr Tower made with a model apparatus constructed on these principles showed him that the best arrangement would be to put a weight on the end of a revolving arm, whereby the centrifugal force of the wave motion might be utilised as well as the rising and falling motion

from *Nature*, **11**, 410, March 25, 1875

choline noise spectra (Katz and Miledi, *J Physiol*, *Lond*, **224**, 665, 1972) On the other hand, using detergent-solubilised receptors, Chiu *et al* (*Biochem Biophys Res Commun*, **51**, 205, 1973) showed that both receptors had the same apparent molecular weight on gel filtration, and Alper *et al* (*FEBS Lett* **48**, 130, 1974) found very similar 'protection constants' for several cholinergic ligands with respect to α-Bgt binding

Many of the α-Bgt binding studies have been done on the intact rat diaphragm but this has certain disadvantages since one is dealing with a slowly diffusing polypeptide which makes kinetic analysis difficult In *Biochemistry* (**13**, 5522, 1974), Almon, Andrew and Appel report some interesting results with Triton-solubilised receptors from rat leg muscles They show ^{125}I-α-Bgt binding isotherms for normal and denervation-induced receptors In both cases they find that the binding follows that predicted for a homogenous ligand interacting with a single class of independent sites They find, however, that the toxin has a 10-fold higher affinity constant in denervated muscle extracts More important, prolonged exposure to 1% Triton X-100 changes the toxin's affinity for the normal receptors to that found for the extrajunctional ones, although the number of sites remains the same

Until now, the separate purification of the two types of muscle receptor has seemed a formidable task, because of the very low yield obtainable from normal muscle Nevertheless, in the Abstracts of the Biophysical Society (*Biophys J*, **15**, 332a, 1975) Brockes and Hall report the purification, by affinity chromatography, of normal and denervation-induced receptors from rat diaphragm Apparently, the two receptors are both glycoproteins and indistinguishable on gel-filtration and zone centrifugation But they do exhibit some differences in affinity for α-Bgt and sensitivity to D-tubocurarine, and show separate peaks on isoelectric focusing

At present it looks as if the normal and denervation-induced receptors are not very different, at least in macromolecular terms The very high density of receptors on the postsynaptic endplate membrane makes interactions between them seem a possible explanation for the lower rate of turnover and other differences observed *in situ* There is, however, no evidence that the solubilised endplate receptors are aggregates of the less densely packed extrajunctional ones On the contrary, the results of Almon *et al* suggest that other hydrophobic components could interact with the receptors in the membrane-bound state, and survive to some extent in mixed Triton micelles on

solubilisation In this context it is interesting that Fambrough (*Neurochemistry of Cholinergic Receptors*, edit by E de Robertis, Raven Press, New York) observed that clusters of toxin-labelled receptors on cultured chick muscle cells could be dispersed by 0 01% Triton, although this treatment did not solubilise the receptors or irreversibly damage the membrane

It seems that to establish the membrane characteristics which are responsible for the stability of the endplate it will be necessary not only to investigate the structure of the receptor protein itself, but also to look more closely at the involvement of other membrane components in the intact tissue

Conservation of nitrogen in climax ecosystems

from Peter D Moore

CLIMAX ecosystems possess several attributes which lead to an increased conservation of essential nutrients, for example, nutrients generally become concentrated in the biota, and nutrient output from the system is low in comparison to this biotic reservoir (Bormann, Likens and Eaton, *Bioscience*, **19**, 600, 1969) In a series of papers, Rice and Pancholy (*Am J Bot*, **59**, 1033, 1972 and **60**, 691, 1973) have now suggested that certain climax vegetation types may inhibit nitrification by soil microbes, as a result of the production of toxins in the litter, thus reducing the rate of nitrate production and loss

In the process of nitrification ammonium ions are oxidised by microbes in two stages to nitrite and then to nitrate, in which form nitrogen is more readily available to the majority of plants In this form, however, nitrogen is easily lost from ecosystems since, being anionic, it is not retained well by clay colloids in the soil Rice and Pancholy therefore regard the suppression of nitrification as a mechanism whereby nitrogen is retained within the ecosystem

They were led to this position initially by a number of reports concerning grassland habitats in which low activities of the nitrifying bacteria and very low nitrate levels were found Theron (*J Agric Sci, Cambs*, **41**, 289, 1951) had suggested that grass roots could secrete a toxin which might affect nitrification and, in African grasslands, Boughey *et al* (*Nature*, **203**, 1302, 1964) suggested that the secretion of toxic substances by the grass *Hyparrhenia* could explain many aspects of the ecology of these habitats Rice and

Pancholy followed up these ideas in an old field succession in Oklahoma by measuring ammonium and nitrate levels in soils from various stages in the succession

They showed high nitrate levels in the early stages falling to low levels at the climax, with ammonium ions behaving in a reciprocal fashion They also found that the density of *Nitrosomonas* and *Nitrobacter* (the nitrifying bacteria) in the soil fell during the course of succession In 1973 they published further data implicating tannins and their derivatives as possible agents in the suppression of nitrification and showed that tannin levels were highest in the climax soils Particularly surprising were the high tannin concentrations in grassland soils and in the litter produced by grasses This, they felt, could account for the low nitrate levels found in many grasslands, particularly since *Nitrosomonas* activity is inhibited by tannin levels of only 2 p p m , less than half the lowest recorded soil concentration in grasslands *Nitrobacter* (which oxidised nitrite to nitrate) was less sensitive, but suppression of one step would effectively block nitrification

Recently (*Am J Bot*, **61**, 1095, 1974) they have attempted the separation and identification of the precise inhibitors which may be involved and, by analysing acetone extracts of plants and soil, have come to the conclusion that many compounds in addition to tannins could be active in suppression, mainly phenolic acids and phenolic glycosides All were more effective in suppressing *Nitrosomonas* than in the inhibition of *Nitrobacter*

Meanwhile, back in the African grasslands, more work has been in progress on the suggested inhibitory properties of *Hyparrhenia*, Purchase (*Plant Soil*, **41**, 527, 1974) has added the washings of this grass to soils and has checked the progress of nitrification After 13 days nitrate production began to fall, but Purchase regards this as the result of increased acidity, since addition of $CaCO_3$ to the water led to continued nitrate production Eluate of the soils seemed not to influence the activity of test *Nitrobacter* cultures, but Rice and Pancholy have shown that the nitrite to nitrate stage of the oxidation is less sensitive to inhibitors Nitrate production was depressed when *Hyparrhenia* root macerate was added to soils, but Purchase puts this down to immobilisation of the mineral nitrogen by the presence of root tissue, since there was no build-up of ammonium ions which one might expect if the nitrifying bacteria were suppressed Purchase considers the scarcity of nitrifying bacteria in *Hyparrhenia* grassland soils to be due to the immobilisation of ammonium ions by root

tissue, hence robbing the microbes of a substrate

Chase, Corke and Robinson (in *The Ecology of Soil Bacteria*, edit by T R G Gray and D Parkinson, 593, Liverpool University Press, 1967) have come to the conclusion that in some soils low pH and phosphate deficiency may limit nitrification Using perfusion techniques they showed that nitrification could be induced in infertile grassland soils, perfused with ammonium sulphate, by the addition of lime and phosphate Purchase (*Plant Soil*, **41**, 541, 1974) has also investigated the possibility that the savanna grassland soils could be deficient in nitrifying bacteria because of low phosphate levels, even when ample ammonium ions are available He added ammonia to soils with varying degrees of phosphate deficiency and found that the nitrification resulting was closely related to the amount of available phosphorus in the soil He concludes that phosphate deficiency in savanna soils could account for the restricted nitrification, the nitrite oxidisers being particularly sensitive It is also possible that the grass roots themselves, together with associated rhizosphere microorganisms, are competing with the nitrifiers for phosphate, thus aggravating the deficiency Robinson (*Plant Soil*, **19**, 173, 1963) has suggested this sort of competition, but for ammonium ions, in New Zealand grasslands

It is now well known that many higher plant species are able to absorb and utilise nitrogen in the form of ammonium ions Recent work by Havill, Lee and Stewart (*New Phytol*, **73**, 1221, 1974) suggests that in some habitats where nitrate production is particularly low, such as acidic, ombrotrophic mires, many of the higher plants have a reduced capacity for nitrate utilisation, since their nitrate reductase activities are very low Such species must depend upon ammonium ions as their major source of nitrogen As Rice and Pancholy have pointed out, this mechanism not only bypasses the microbial nitrification stage, which may reduce the risk of nitrate loss from the ecosystem, but also saves the plant the energy required for the activity of nitrate reductase Whatever the mechanism whereby nitrification is suppressed in some ecosystems, and it is likely that the precise nature of the suppression varies from one ecosystem to another, the resulting nitrate scarcity has evidently placed a selective pressure upon the resident plant species, resulting in their ability to use ammonium ions This evolutionary response and the suppressed nitrification which has engendered it, may well provide a mechanism for ecosystem nitrogen conservation

articles

Geology, fauna and palaeoenvironments of the Ngorora Formation, Kenya Rift Valley

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The Ngorora Formation occurs within a long sequence of stratigraphic units that are firmly calibrated by $^{40}K/^{40}Ar$ dates It contains an impressive fauna and has yielded a valuable amount of information on local palaeoenvironments and on the sedimentary processes operative during its deposition Here, a previously unpublished vertebrate and invertebrate faunal assemblage between 9 and 12 Myr old is recorded, and the stratigraphic framework of this former gap in the fossil mammalian record of Africa south of the Sahara is described

RESEARCH on the Ngorora Formation[1-7] has produced a detailed but as yet incomplete picture of the palaeoenvironments and fauna Key marker horizons have been traced from area to area, detailed measurements have been made of numerous well exposed sections and facies variations have been recognised over a large area Of particular faunal interest are the collections of Equidae, Hippopotamidae, Orycteropodidae, Hyaenidae, Hyracoidea, Gomphotheriidae and Canidae Antlered ruminants similar to forms from Fort Ternan have been found, and remains of a small listriodont suid are relatively common

Palaeogeomorphological setting

The Ngorora Formation was deposited in a basin floored by rocks of the Tiim Phonolites Formation, and was limited to the west by the Elgeyo Escarpment, to the east by a rise towards the area of the present Laikipia Plateau and to the north by the uplands round the Tiati volcanic centre To the south, the boundary was controlled by the rise of the rift floor, in a similar way as at present (Fig 1) The basin was tectonically active before, during and after the deposition of the Ngorora sediments The principal structural trends within the basin are exhibited by escarpments along the Kito Pass, Saimo, Cherial and Kaption faults

The Saimo Horst was a topographic high during most of Ngorora times and underwent contemporaneous weathering and erosion for at least part of the time The sediments thin rapidly north-eastwards towards Sidekh, which was also a strong positive element in the topography and is unlikely to have been completely covered by sediment or water during the deposition of the Ngorora Formation

These two upland areas were separated by about 8 km of low lying country near Bartabwa (C5, Fig 2) which was mantled by an extensive cover of sediments (Fig 2) The Ngorora Basin may be divided into three areas on the basis of the types of sediment they contain (Fig 1) Area I, which lies to the north of the Saimo Horst, comprises two subdivisions The first is the type area of Kabarsero (area I*a*)

lying to the east of the Kito Pass Fault The second area lies on the western, upthrow side of the fault, where the sequence is thinner (area I*b*) Area II comprises and the slope of the Tugen Hills west of the Cherial Fault and contains the Kapkiamu Graben and the Kaption Volcanic Complex Area III lies to the south of the Saimo Horst and contains the upper part of the Ngorora succession lying on a deeply weathered surface of Tiim Phonolite Correlations between the three areas must remain tentative because of lack of continuity of outcrop between them and the absence of marker horizons common to them all

Stratigraphy

The thickness of the Ngorora Formation varies greatly in different parts of the basin At Kabarsero, the type area, the sediments are 400 m thick whereas in area I*b*, at Kalimale they are 215 m thick (Fig 3) The formation thins rapidly north-eastwards from Kalimale (C6, Fig 2) and pinches out below the summit of Sidekh In the south, in area III, only the upper parts of the formation are present, and these thin southwards and pinch out at about 0°30'N

Bishop and Chapman[1] divided the formation into five units which are here ranked as members (Fig 3) The lowest unit, member A, consists in area I of thick beds of coarse volcaniclastic sediments and clays Some of the beds originated as lahars, possibly from the Kaption Volcano, lying in area II to the south-west

Member B consists of alternating, current bedded, gritty tuffaceous beds, clays and silts An accretionary lapilli tuff in area I may be correlated tentatively with a very thick unit of similar lithology in area II On the upthrow side of the Kito Pass Fault (area I*b*) the clays and silts found in the type area are absent and beds of sandy and gritty tuffs lie directly on top of one another

In area I*a*, member C is composed of finely laminated clays and shales with occasional sand grade beds intercalated On the upthrow side of the Kito Pass Fault (area I*b*) the latter part of member C is cut out by slight erosion and channelling In area II over 200 m of alternating shales and laminated clays are found in the Kapkiamu Graben The shales consist almost entirely of chemical precipitates, and the clays were probably produced largely by weathering of the Tiim Phonolites In area III an unknown thickness of diatomaceous suncracked shales overlies a weathered surface of Tiim Phonolites, but it is not known whether these are the lateral equivalent of member C

In areas I and II, member D is a succession of clays rhythmically alternating with cross-bedded silty and gritty tuffaceous beds Earth movements during the deposition of member D are indicated by slumps, faults and fissures which do not affect the overlying units At Cheprimok (C5, Fig 2) major faulting resulted in the erosion of nearly 130 m of
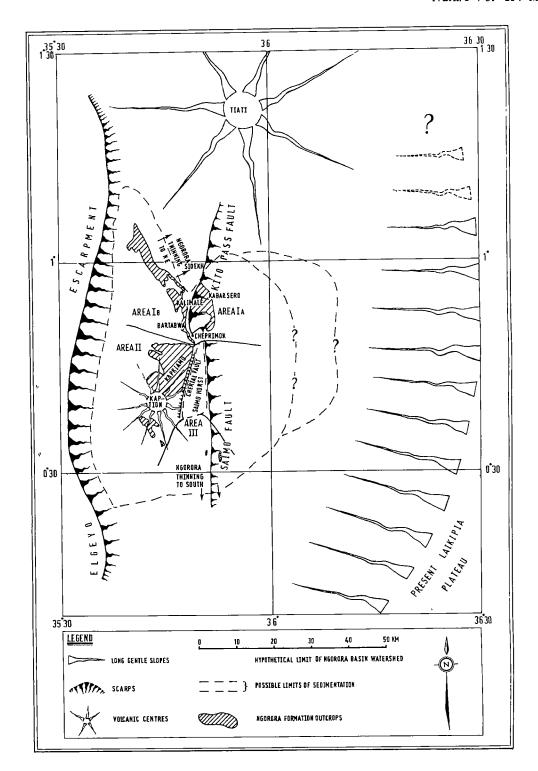
Fig 1 Regional setting and outcrops of the Ngorora Formation

sediment so that member E rests unconformably on member A and oversteps on to Tiim Phonolite This faulting episode was widespread and has been recognised in area II, 20 km away In area III a series of red marly earths and silts may be the lateral equivalent of member D

Member E sediments were deposited after another major faulting episode which lowered the base level to such an extent that lacustrine conditions were established again A series of diatomaceous and fish-bearing shales is widespread over the whole of area I and parts of area II, but erosion has removed much of the record The erosion was consequent on movement on the Kito Pass and Saimo Faults which uplifted the crest of the Tugen Hills tilt block until it was exposed to subaerial processes Area Ia, on the down-

throw side of the faults, underwent further sedimentation but the changed environment resulted in the deposition of coarse material including conglomerates, rather than the deposition of shales like those found in the lower part of the sequence In area III, a series of conglomerates and bedded tuffs may be equivalent to member E In every area an erosional unconformity separates member E from the overlying Ewalel Phonolite Formation

Figure 3 illustrates stratigraphic sections from areas Ia and Ib respectively, to show variation in thicknesses of the different members Several important horizons can be traced across the Kito Pass Fault and reveal thickness changes indicating contemporary fault movement throughout the deposition of the sequence The units on the upthrow side

f the fault are usually condensed sequences, whereas
1ose on the downthrow side often contain extra units of
ays and silts

Sediments are derived from several sources The Kaption
olcano in area II probably supplied many of the lahars
1d much of the tuffaceous material found in area I Some
diment was derived from the Laikipia area and also from
e Tiati volcanic centre The laminated clays of the
apkiamu Graben may have been derived from phonolite
eathering products on Saimo Horst

The Saimo Horst and Sidekh tilt block acted as barriers
sediment derived from the east, although the Bartabwa
ip may have functioned as a funnel for sediment crossing
e Kito Pass Fault Channel deposits in area Ia to the east
' the line of the Kito Pass Fault are more fossiliferous
86 specimens from 19 localities) than those in area Ib to
e west (59 specimens from 11 localities)

auna and faunal correlation

n extensive collection of fossils was made during the
72–73 field season It included 1,400 fragments of mam-
als, 500 fragments of fish, 3,000 mollusca and a variety
less common fauna and flora, from 132 fossiliferous
calities Many of the specimens have not yet been studied
detail but preliminary identifications are presented in the
unal list (Table 1) Figure 4 is a pictorial representation
the stratigraphic ranges of the various faunal and floral
:ments

A tentative correlation with the uppermost Vindobonian
s been attempted[3] on the basis of the fauna and particu-
·ly on the apparent absence of Hipparion The discovery
Hipparion in members D and E resolves much of the
:culation over the apparent absence of Equidae from sub-
haran Africa at this early date Hooijer[8] has concluded
it the Ngorora Equidae are the same species as the
rliest equids of northern Africa, and Eurasia

The Ngorora Formation is younger than the Fort Ternan
rmation from the evidence of radiometric dates and mam-
ilian faunas The Bovidae seem slightly more advanced
in their Fort Ternan counterparts (A Gentry, personal
mmunication) A Vindobonian age is clearly too old and
: Ngorora Formation is probably equivalent to the Val-
ian and Lower Turolian of Europe and possibly the
inji/Nagri Stages of the Siwalik Series

When additional dates have been established for tuff
rizons in the sediments it should be possible to estimate
ire precisely the age of these earliest sub-Saharan Equidae
present, whole rock $^{40}K/^{40}Ar$ age determinations suggest
:s of ~12 Myr for the underlying Tiim Phonolite and
; 5 Myr for the overlying Ewalel Phonolite[2,6] Sanidine
dspars from member B have yielded ages of ~11 94 and
2 31 Myr and ages of ~9 82 and ~9 68 Myr from
:mber D (J A Miller, personal communication)

The oldest known remains of Hippopotamidae occur in
: Ngorora Formation[2] The number of specimens has
:n significantly increased by finds during the latest field
ison Other interesting elements of the fauna include a
minoid, a cercopithecoid, a new genus of large hyracoid,
new species of gomphothere[7], a canid, a mellivorine
istelid, a new species of Orycteropus, the ruminants
itragocerus, ?Pseudotragus, Palaeotragus, Gazella, ?Samo-
rium, and an antlered form, several rodents and a small
riodontine suid A hyaenid has also been recorded A
ge variety of molluscs has been collected, and impressions
several types of insect have been found, including ter-
tes, mosquitos, moths, and flies Of striking interest is the
:at difference between the rhinocerotid faunas of Ngorora[4]
d those of Fort Ternan This is strange in view of the
nilarity between the ruminants found at both localities
Several earlier determinations of taxa are incorrect
alicotheriidae[1] is now recognised as Hyracoidea
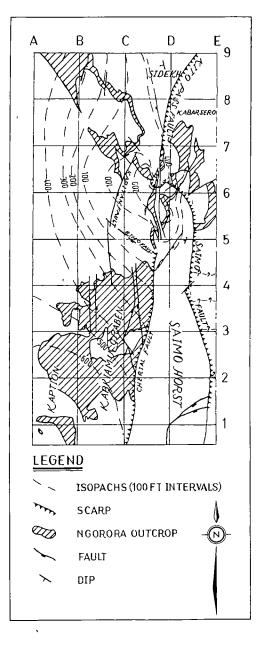
Fig 2   Thickness variation and major structural controls,
Ngorora Formation

Chilotherium[2] should be Chilotheridium[4] Tapir[3] should be
Listriodontinae, and Deinotherium hobleyi[2] should be Deino-
therium bozasi

Various plant fossils have been collected, including wood,
leaves and seed pods Among the Protista the diatom
Melosira sp is recorded, and calcareous algae are common
Among the Trachaeophytes, both Monocotyledons (palms
and grasses), and Dicotyledons (lianas, ?Celtis sp) and
several others are represented A bullrush-like plant was
found in member B No pollen has been found

The Ngorora fauna is not restricted to a single small
locality as is that at Fort Ternan, but occurs over a large
area It spans a period possibly as long as 3 Myr Its
value is enhanced by the detailed palaeogeomorphological
and palaeoenvironmental evidence that can be deduced for
the formation (Fig 4 and Table 2)

Palaeoenvironment

Reconstructions of palaeoenvironments often rely heavily on
fossils, and thus engender circular arguments about the
habitats occupied by different elements in the fauna Apart
from details of the topography of the basin floor already

KABARSERO KALIMALE

EWALEL FORMATION

EWALEL FORMATION

MEMBER 'E' 125m

MEMBER 'D 66 5m

MEMBER 'C' 62m

MEMBER B 42 5m

MEMBER 'A' 104 m

E2

E1

D1

C1

B4
B3
B2
B1

A5

A4

A3

A2

A1

MARKER HORIZONS

KITO PASS FAULT

E2 uc

E1

D1

C1
B4
B3
B2
B1

A5

A4

A3
A2
A1

75 + m

16 m

9 m

25 m

90 m

TIIM FORMATION

TIIM FORMATION

KEY

Channel deposit	
Sand	
Silt	
Clay	
Shale	
Clastic dyke	
Palaeosol	
Cross bedding	
Accretionary lapilli	
Tuffaceous boulders	
Agglomeratic base	
Sun cracks	
Fossil locality	
Leaves	
Fish	
Lava	
Manganiferous horizon	
uc Unconformity	

Table 1 Faunal list from the Ngorora Formation

Mollusca		Primates	
Gastropoda		Cercopithecoidea	indet
Prosobranchia	*Lanistes* sp *	Hominoidea	indet
	Bellamya sp *	Rodentia	Dendromurinae gen nov *
	Melanoides tuberculata small sp *		Cricetidae gen nov small* gen nov medium*
Pulmonata	sp indet *		Phyomyicae gen nov *
Bivalvia	*Pleiodon* sp *		?Pedtidae*
	Mutela? sp *		?Sciuridae*
Annelida	indet *	Carnivora	
Arthropoda		Canidae	gen nov ?
Insecta		Mustelidae	*Mellivora* sp nov *
Isoptera	indet *	Hyaenidae	*Percrocute tobieni* Crusafont
Lepidoptera	indet *		& Agurre
Diptera	indet *	Tubulidentata	
Coleoptera	indet *	Orycteropodidae	*Orycteropus* sp nov *
Crustacea		Proboscidea	
Ostracoda	*Heterocypris* sp ?*	Gomphotheriidae	*Gomphotherium ngorora* Maglio
	Candona sp *	Deinotheriidae	*Deinotherium bozasi* Arambourg
	Metacypris sp *	Hyracoidea	
	Limnicythere sp *	Procaviidae	*Paraphiohyrax* sp nov
Decapoda	*Potamon* sp *	Perissodactyla	
Pisces		Equidae	*Hipparion primigenium* von Meyer
Cyprimformes		Rhinocerotidae	*Chilotherdium pattersoni* Hooijer
Cyprinidae	indet *		*Aceratherium* or *Diceroihinus*
Clariidae	c f *Clarias* sp		*Brachypotherium* sp
Perciformes			
Cichlidae	*Tilapia* sp nov	Artiodactyla	
Reptilia		Suiformes	
Chelonia		Suidae Listriodontinae	gen et sp nov *
Testudinidae	*Testudo* sp *	Hippopotamidae	c f *Hippopotamus* indet
Trionychidae	*Trionyx* sp		gen nov indet
Pelomedusidae	*Pelusios* c f *sinuatus* Smith	Ruminantia	
Crocodilia		Tragulidae	2 spp
Crocodilidae	*Crocodylus* sp	Giraffidae	*Palaeotragus* sp
Squamata			?*Samotherium* sp *
Varanidae	indet *	Lagomerycidae	indet
Ophidia	indet *	Bovidae	*Protragocerus* sp
Aves			Boselaphini/Tragelaphini sp or spp
Struthioniformes			?Cephalophini sp
Struthionidae	indet *		?*Pseudoragus postpotwaricus* Gentry
Ciconiiformes			Neotragini sp *
Ciconiidae	*Leptoptilos* sp		*Gazella* sp
Mammalia			
Insectivora			
Macroscelididae	indet *		

*Previously unrecorded from the Ngorora Formation

discussed here, the Ngorora sediments yield evidence of a variety of conditions prevailing both during and after deposition Palaeoenvironmental indicators in the sediments include polygonal desiccation cracks, calcretes and other palaeosols, in situ plant remains, channelling and cross-bedding, bird footprints, evaporite deposits, rhythmic units, algal and oolitic limestones, subaerial tuffs, diatomites, chert nodules and other structures Table 2 summarises the evidence for the five members in three different areas

Member A predominantly comprises coarse, reworked pyroclastic material, with some primary elements (for instance around the Kaption volcanic centre) The coarse beds contain very few fossils, but the thin shaly horizon at the bottom of the type section contains rhizomes in position of growth At Chepchomus (C6, Fig 2), a few proboscidean and rhinocerotid remains were found, resting on the Tiim Phonolite At the top of member A some grit-filled channels occur, but these were poorly fossiliferous and yielded only a few reptilian fossils together with comminuted fish bones

During the deposition of member B, Kaption Volcano

◄Fig 3 Lithostratigraphic units and sedimentary characteristics, Ngorora Formation

was still intermittently active and was quite possibly a source of the accretionary lapilli tuff, one of the most reliable marker horizons in the Ngorora sequence In area 1b, there were several episodes of emergence of the land surface giving rise to palaeosols and weathering profiles In particular, there is a widespread pyrolusite nodule horizon in the Bartabwa area (C5, Fig 2) which seems to have been formed by subaerial weathering of the underlying tuffaceous bed It was subjected to contemporaneous channelling and winnowing, as evidenced by thinning of the unit and local concentration of the nodules into a conglomerate The accretionary lapilli tuff was deposited after the formation of the pyrolusite horizon, and soon acquired an active and flourishing plant growth, as shown by numerous rootlet casts In places, as at locality 2/1 (Kabarsero), it was channelled and potholed by running water A mile south of Bartabwa, casts of rhizomes occur in the accretionary lapilli tuff and at Kalimale (C6, Fig 2) plant growth and weathering were vigorous enough to destroy all the lapilli in a bed 45 cm thick, giving rise to an extensive calcrete (caliche) horizon Fortunately, patches of the original material have survived, thus allowing stratigraphic correlation

The varied fauna recorded from member B is of limited
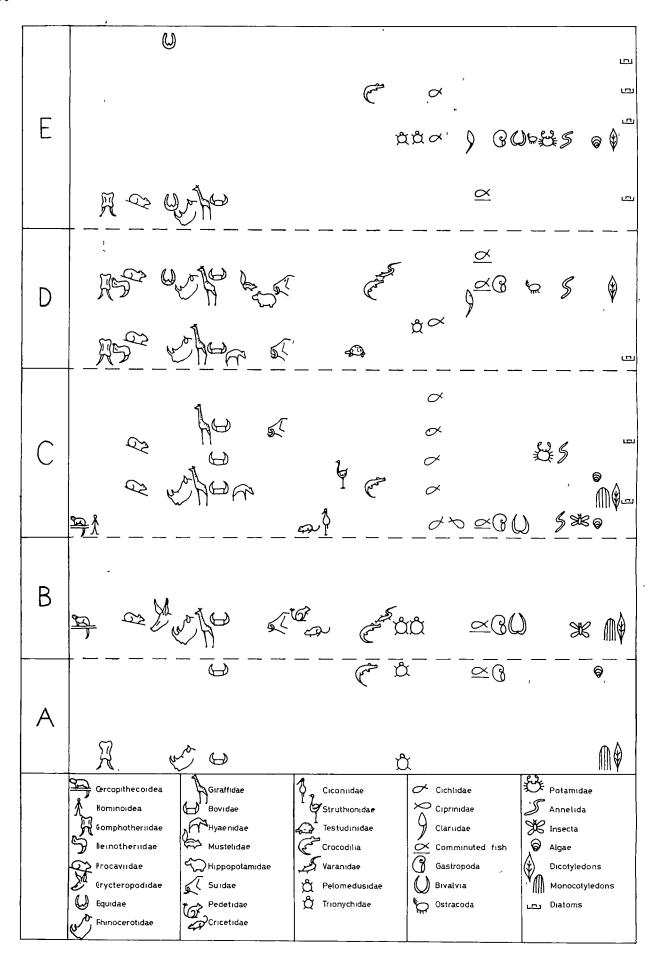
Fig 4 Distribution of major faunal elements, Ngorora Formation

use in deducing the nature of the local palaeoenvironment, as nowhere is there a 'life assemblage'. It could have been derived from many miles away, and from several different environments. The ruminants, however, indicate an open or lightly wooded grassland habitat[5].

Kaption Volcano had ceased activity by the beginning of deposition of member C which was heralded by large scale faulting along both the Kito Pass–Saimo Fault system, and the Cherial–Kaption system. Three lakes were formed, one at Kabarsero (area I*a*), one in the Kapkiamu Graben (area II), and the third to the west of the Kaption Complex. The first was a fresh water lake, as shown by the diatoms and crabs, and could have been well oxygenated. Several regressions occurred and the sediments became suncracked and fissured, with the development of palaeosols. Some of the palaeosol horizons have yielded fossils, including articulated bones and, in one case, a complete skeleton. These occurrences are more likely to represent life assemblages than are those of members B and D. Taphonomic studies are being undertaken to determine the main differences between the two types of deposit. Potamid crabs indicate that the water of the Kabarsero lake was fresh. The Kapkiamu lake on the other hand was most probably an alkaline body of water; the shales were probably chemical precipitates (Judith Van Couvering, personal communication). A new form of stunted *Tilapia* is found in abundance in the shales, and is similar to *Tilapia grahami* found at the present day in the soda lake Magadi (Judith Van Couvering, personal communication). Beds of algal and oolitic limestone that give off a petroliferous odour when freshly broken also occur. They usually overlie suncracked horizons.

The shales and laminated clays were laid down in rhythmic fashion and possibly indicate seasonal fluctuations of climate. Both units are finely laminated, and record periodic increments of sediment. Fossil wood from member C contains well developed growth rings suggestive of fluctuating climatic conditions.

The juxtaposition of fresh and saline lakes comes as no surprise as there are two such systems in existence in the Kenya Rift Valley at the present time. Lakes Baringo and Hannington lie about 20 km apart and lakes Naivasha and Elmentaita lie about 25 km apart. The palaeolakes of Kabarsero and Kapkiamu were about 8 km apart. The area between them was one of low ground upon which a vigorous plant cover and palaeosols were developed. Contemporaneous erosion was occurring and in places channels are seen, but the relief was, in general, low. Some manganiferous weathering horizons were developed during this time, and it is above one of these, at locality 2/9 (C5, Fig. 2), that the hominoid molar was found. The third lake, west of Kaption, contained freshwater.

Member D began with rejuvenation which resulted in channelling, and the deposition of grits and conglomerates and coarse tuffaceous beds. There is a marked decrease in grain size to the west. Clay horizons are intercalated between the coarse units in a manner similar to those of member B. Unfortunately, landslip and scree obscure much of member D in area I*b*, but in area II there are several good exposures which contain suncracked horizons, palaeosols and other evidence of emergence or shallow water. Crabs, ostracods and fish are found at Kalimale (C6, Fig. 2) in a marginal lacustrine deposit, indicating freshwater conditions nearby.

Scree and landslip obscure much of member E in area I*b*, and in area II the unit has been eroded away almost completely. The lower part of the member is composed of well laminated, diatomaceous shales with numerous suncracked layers. The development of clastic dykes and 'sills' is in places spectacular. At Cheprimok (C5, Fig. 2) sedimentary rhythms are well developed with up to nine units comprised of a coarse volcaniclastic and fish-bearing lower part, followed by a shaly, suncracked portion. Zeolite formation is common and palaeosols are developed on some units. One

of these palaeosols contained the remains of a new gomphothere[7]. The lacustrine episode of member E was terminated by renewed movement along the faults which resulted in the lowering of area I*a* and the uplifting of areas I*b* and II which began to undergo erosion. In area I*a* sedimentation was continuous, but the energy of the environment had changed so that grits and conglomerates were deposited above the shales.

Table 2 Summary of palaeoenvironmental reconstructions, Ngorora Formation

Member	Area I*a*	Area I*b*	Area II
	Rejuvenation, giving rise to conglomerates and grits.	Uplifted by faulting with consequent erosion.	
E	Lacustrine, fresh water with numerous lake oscillations producing suncracks and clastic dykes.	Lacustrine, fresh water, well developed rhythmic units caused by lake oscillations. Some calcretes formed.	Record removed by erosion.
D	Fluviatile with several prolonged emergences marked by palaeosols. Ruminants suggest open or lightly wooded areas.	Marginal lacustrine and fluviatile; fresh water. Fluctuating lake levels with frequent desiccation.	Lacustrine environment with minor fluviatile episodes. Some palaeosols and desiccation cracks developed.
C	Freshwater lacustrine environment. Oscillating lake levels with prolonged emergences marked by palaeosols. Growth rings in wood suggest fluctuating climate.	Predominantly emergent with weathering and erosion. Minor paludal facies. Low relief.	Alkaline lacustrine conditions with very low energy. Many lake level fluctuations. Rhythmic units developed, with possible climatic control.
B	Fluviatile with some prolonged emergences and development of palaeosols. Possibly two primary tuffs. Vigorous plant growth.	Predominantly emergent with well developed palaeosols. Otherwise fluviatile. At least one primary tuff. Vigorous plant growth.	Little evidence, owing to proximity to volcanic centre. Thick primary tuffs.
A	Lacustrine at first followed by several lahars, primary agglomerates and tuffaceous sediments. Minor fluviatile facies.	Lahars, tuffaceous sediments; minor fluviatile facies.	Primary volcanic activity, near eruptive centre; tuffs, agglomerates and lavas.

The sedimentary sequence is overlain by lavas of the Ewalel Phonolite Formation which flowed north and covered much of the Ngorora Basin. Continued faulting along the Kito Pass and Saimo Faults, and later erosion led to re-exposure of the sediments. The area was then covered by the Kabarnet Trachyte Formation, K–Ar dated at about 7 Myr (ref. 2). Further faulting, in particular during the Pleistocene, resulted in the uplift of the Tugen Hills to their present height, and re-exposed the Ngorora Formation.

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Structure of tobacco mosaic virus at 6.7 Å resolution

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The electron density distribution of tobacco mosaic virus has been determined to 6.7 Å resolution by analysis of the X-ray diffraction pattern given by oriented gels of the virus. This has been achieved by separation of overlapping Bessel function terms by a technique analogous to crystallographic isomorphous replacement. The course of the polypeptide chain of the coat protein may be traced for a large part of its length.

TOBACCO mosaic virus (TMV) consists of long, rod-like particles which can be made to form large regions of oriented gel in a capillary tube. These gels give a detailed diffraction pattern (Fig. 1), as was shown by Bernal and Fankuchen[1]. This demonstrates that TMV is periodic, repeating every 69 Å with a pseudo-repeat every 23 Å. The theoretical development by Cochran, Crick and Vand[2] of the diffraction pattern of a helix allowed Watson[3] to recognise that the diffraction pattern given by TMV gels was a typical helix diffraction pattern and moreover that the repeating unit of TMV must contain $3n + 1$ subunits in three turns. It was later shown by Franklin and Holmes[4] that there are 49 subunits in three turns. Franklin[5] and Caspar[6] independently used the method of isomorphous replacement applied to the zero layer line of TMV to calculate a radial density distribution of the virus, and Franklin[5] showed that the RNA is at a radius of 40 Å. Klug (unpublished) later confirmed that this is a single strand winding along the basic helix, by studies of the other layer lines.

Because the TMV particles are randomly oriented about their long axes, one obtains in a diffraction experiment the cylindrical average of the square of the Fourier transform of a single virus particle. Interparticle interference may be neglected, so the intensity is continuous. Franklin and Klug (unpublished) demonstrated that it should be possible to extend the method of isomorphous replacement as classically used in protein structure analysis to deal with the situation encountered in TMV. A number of theoretical and technical problems impeded the progress of this research and our paper represents the fruition of this attempt to determine the structure of TMV from oriented gels using the method of isomorphous replacement.

Major sources of difficulty

The amount of data available is limited and errors tend to be systematic, so that extremely accurate measurement and correction of data is required.

The main problem encountered concerns the location of heavy atoms. Because of the very high symmetry of TMV (49-fold) and the low resolution data available, Patterson methods were early shown to be impractical. Automatic search methods based on R-factor minimisation using single heavy atom derivatives gave ambiguous results. The use of double heavy atom derivatives together with the single heavy atom

derivatives can partially remove these ambiguities[7] but a full scale R-factor search using five heavy atom derivatives and two double derivatives, and more accurate data was required in this work to yield unambiguous heavy atom positions. These methods are described briefly below.

The problem of cylindrical averaging is solved in two stages. At low resolution (10–15 Å) the diffraction intensities are unaltered by the averaging, because of the high symmetry of the TMV particle. Therefore it is possible to reconstruct the electron density of virus by establishing the phases of the observed intensities by the normal method of isomorphous replacement. At higher resolution new methods have been required, based on an extension of the isomorphous replacement method.

Data collection

Data were collected with a double-focused monochromatised beam (see Fig. 1) and a computer-controlled densitometer. The major corrections were background subtraction and a correction
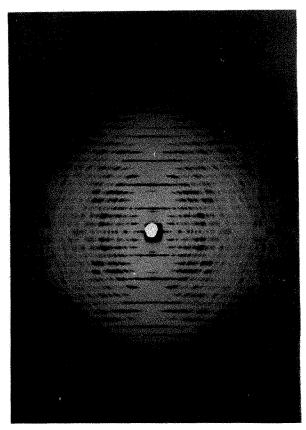
Fig. 1 A fibre diffraction pattern from an oriented gel of TMV. The exposure time was 87 h in an evacuated Guinier focusing camera (specimen film distance 11.2 cm) using a point focused beam from two bent quartz focusing monochromators and an Elliott fine focus rotating anode tube.

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Table 1 Symbols used in this paper

R, Reciprocal space radius
l, Layer line index
c, Axial repeat (69 Å in TMV)
r, Real space radius
φ, Real space azimuthal angle (cylindrical coordinates)
z, Real space vertical coordinate
f, Atomic scattering factor
F, Structure factor
J_n, Bessel function, order n

for disorientation. Automatic background subtraction had not been successful, because arcs from strong spots extend into the region between layer lines where background is measured. But an interactive computer display system using manual intervention where necessary to override automatic procedures has proved adequate. These and other developments are described by Barrington Leigh[8] and Mandelkow[9]. The correction for disorientation has been described by Holmes and Barrington Leigh[10] and Stubbs[11].

Location of heavy atoms

A summary of previous attempts to locate the heavy atoms is given in ref. 7. The use of single site substitutions was of paramount importance in the initial location. The heavy atoms were located in three stages:

Determination of r and f. Radius and occupancy are the only parameters which affect the intensity on the inner centric part of the zero layer line. They may be determined by comparing the difference between the native and derivative structure factors (G) with the expected structure factor of a heavy atom ($fJ_0(2\pi Rr)$). The parameters are refined by a least squares procedure. An exact description of the process is given by Mandelkow and Holmes[15].

Determination of φ and z. It can be seen from equation (2) that, for a given Bessel function term, the phase Φ of the contribution of one atom to G is a constant

$$\Phi = -n\varphi_j + 2\pi l z_j/c$$

where r_j, φ_j, z_j are the cylindrical polar coordinates of the heavy atom. Φ may be found by a search procedure[8], in which the least-squares R-factor for each layer line

$$R = \sum (|F_H|_{obs} - |F_H|_{calc})^2$$

is minimised with respect to Φ. The values of Φ found from different layer lines can be combined to find φ_j and z_j. The procedure is limited to the regions of the diffraction pattern where Gs do not overlap.

Refinement. Refinement analogous to conventional crystallographic heavy-atom refinement[16] was carried out on the heavy atom r, φ and z coordinates by an extension of the process outlined above. There were insufficient data to refine occupancy or temperature factor.

Cylindrical averaging

The structure factor of a helix of repeating subunits was first evaluated by Cochran, Crick and Vand[2]. From their results, Waser[12] and Franklin and Klug[13] calculated the cylindrically averaged intensity. In the notation of Klug, Crick and Wyckoff[14] this is

$$I = \sum_n G_{nl} G^*_{nl} \tag{1}$$

where

$$G_{nl} = \sum_j f_j J_n(2\pi R r_j) \exp i(-n\varphi_j + 2\pi l z_j/c). \tag{2}$$

The summation in equation (2) is over all the atoms in the asymmetric unit. The notation is given in Table 1.

The summation over n in equation (1) is confined to those values of n which obey the helix selection rule

$$l = tn + um$$

where the helix has u subunits in t turns (49 and 3 for TMV); m is any integer. Since a Bessel function has a very low value until the argument is nearly equal to the order only a small number of different orders (and thus G terms) can contribute to the intensity at low resolution. For TMV to a resolution of about 12 Å, there is only one term, and two terms suffice for a resolution of 6–7 Å.

To perform a Fourier inversion and thus obtain an electron density map, the real and imaginary parts of each G must be known. The problem may be formulated in a way analogous to the classic crystallographic phase problem but with three unknown parameters rather than one. These may be considered as the phase of each of two terms originating from different
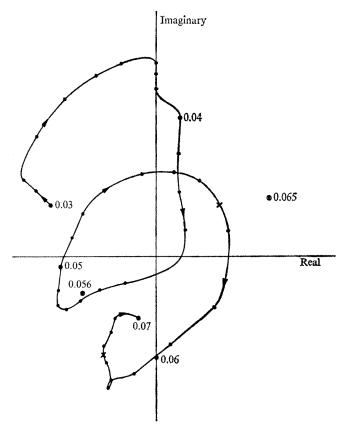
Fig. 2 Continuity of the transform of TMV, for layer line 1, $R = 0.030$ to 0.070. Each dot represents the value of the most probable G on the Argand diagram. The line connects dots adjacent in R. Circled dots do not fit the general continuity. The crosses represent minor peaks in the probability distribution of such discontinuous points, which do fit the continuity. The points represented by crosses are therefore accepted as the value of G to be used, rather than the circled dots. Argand diagrams such as this have previously been used by A. Klug (personal communication).

order Bessel functions (see equations (1) and (2)) and the ratio of their amplitudes, which for convenience is often expressed as the tangent of a third angle. The problem is equivalent to searching over the surface of a four-dimensional hypersphere using four or more heavy atom derivatives to determine the correct hyperspherical (three component) phase angle. In practice, however, it is more convenient to determine the G parts directly. They can be found by the method of isomorphous replacement. If a heavy atom is bound to the structure, and its position is known (see below), its contribution to G can be

Table 2 Heavy atom derivatives

Ligand	Strain	Residue	f	$r(\text{Å})$	$\varphi(°)$	$z(\text{Å})$
DMA	*Ni*-2068	139	40	72	0	0
MMN	*Vulgare*	27	32	56.7	16.0	11.7
OsO₄	*Vulgare*	1?	35	92.5	5.7	9.4
SHIMS-MMN	*Vulgare*	68(+27)	25	71.1	5.3	−1.3
Pb²⁺	*Vulgare*	115/116	24	25.4	7.1	20.6
Double derivatives (excluding SHIMS)						
DMA/Os	*Ni*-2068	139/1?				
MMN	*Ni*-2068	139/27				

SHIMS, sulphydryl imidoester[27]; DMA, dimercury acetic acid; MMN, methylmercury nitrate[4]. Units of f are arbitrary, and correspond to approximately one-third of an electron.

calculated. If this is $a+ib$, and $G = A+iB$, we have from equation (1)

$$I_H = \sum (A+a)^2 + (B+b)^2 \qquad (3)$$

To solve unambiguously for all As and Bs, as many heavy atom derivatives are required as there terms in the summation in equation (3). The solution may then be found numerically, by a procedure quite analogous to conventional phase determination. This requires a great deal of computing, however, so an analytical procedure has been developed (Diamond and Stubbs, unpublished, based on the usual assumption that there are no errors in native intensities. With this assumption a series of equations of the type (3) may be reduced to linear equations, errors in which are minimised subject to the non-linear constraint implied by equation (1). The results will usually take the form of the peaks of a probability distribution for the pairs (A, B). It would be quite possible to accept the most probable set of As and Bs, treating each measured point on the fibre diagram separately. But to do so would be to neglect an important constraint on G.

The continuity constraint

As mentioned above (equation (2)), G is a continuous function of R, and the restriction $r_{max} < 90$ Å (the maximum radius of the particle) puts a limit on the rate of fluctuation in G. If, for example, a series of points along a layer line have smoothly varying values of A and B, except for one point, a smaller peak in the probability distribution of the discontinuous point often corresponds to the value of the smooth curve. This is illustrated in Fig. 2 for the single Bessel function case. The more compli-

cated situations which arise when two Bessel functions are present can be dealt with either in the same way (by inspection), or by a semi-quantitative procedure suitable for a computer (Stubbs, unpublished). Thus, real and imaginary parts for each G can be determined which provide an optimum balance between probabilities based on isomorphous replacement data and the constraint of continuity.

The derivatives
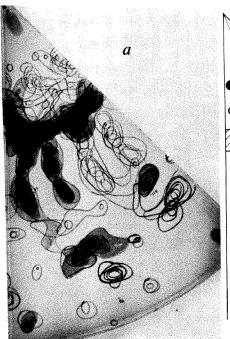
Four single-site derivatives and three double derivatives, with a total of five independent sites were used. The double derivatives were originally used to assist in the location of heavy atom sites. In this work, their main function was to reduce errors by providing partially independent extra data, but in the last few rounds of refinement, the methylmercury nitrate site at residue 139 (see Table 2) was freed from the dimercury acetate site. The movement was in fact very small.

Table 2 summarises the information on the derivatives. Because of the importance of single sites, sulphydryl-mercury reactions were used whenever possible. The *Ni*-2068 mutation[17] was used because it contains only one sequence change, Tyr 139 → Cys. The large dimercury acetic acid (DMA) group can react with this residue, but not with Cys 27, which only reacts with small mercurials.

The osmium site is tentatively assigned to the N-terminus, because it is the same as the N-terminus of the strain U2 of TMV[15]. This strain differs from *vulgare* in 35 amino acids of the coat protein, but gives a very similar diffraction pattern. The assignment is made plausible by the fact that a difference Fourier between TMV-*vulgare* and U2 shows no significant differences at radii greater than 70 Å. The chemical location of the lead site is uncertain. Butler and Durham[18] concluded that the reaction, which is believed to be with carboxyl groups (see ref. 19), could only be with Asp 115 and Asp 116.

The electron density map

Apart from a short section of layer line 3 where there are three G terms (and this section has relatively low intensity so that it could be omitted), only one or two G terms contribute to the data to a resolution of 6.7 Å and we were able to evaluate the A and B parts of each G term within this region. This was done
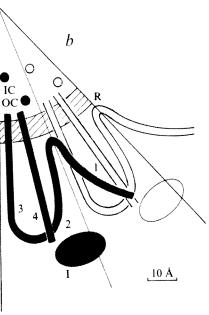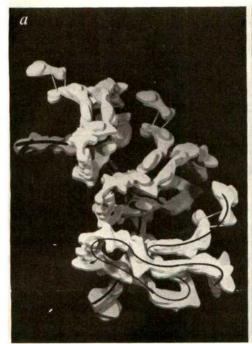
Fig. 3 *a*, Five sections of the electron density map, at 2 Å intervals in *z*. The sections contain two molecules adjacent in φ. One molecule and the RNA strand are shaded. Clearly visible are the RNA, parts of ribbons 2 and 3 (see Fig. 4*b*) and the C-terminus region. *b*, Schematic diagram of the main features in the electron density map. The diagram represents two subunits, one being shown by open symbols for convenience. IC, Inner column; OC, outer column; I, island; R, RNA; 1, 2, 3, 4, ribbons.
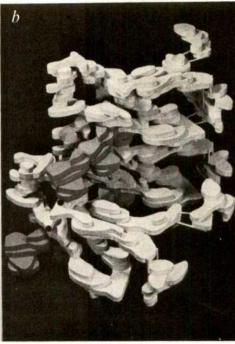
Fig. 4 Balsa wood models of the electron density. *a*, A half circle of RNA is shown (heavy line) with three protein molecules arranged along the TMV helix. Every other subunit has been omitted for clarity. The main chain of the protein is shown (light line). *b*, Three subunits of TMV protein, showing the interaction between subunits in successive turns of the helix. Note the three points of RNA-protein interaction, which are marked (●).

by trapezoidal integration at intervals of 0.001 Å^{-1} along each layer line. Compared with the mean wavelength of the *G*s 0.001 Å^{-1} is a fine interval. A Fourier inversion was computed. Five sections of the resultant electron density map are shown in Fig. 3*a*. Figure 3*b* is a summary of the main concentrations of electron density. The very strong helical ribbon at about 40 Å radius may be identified as RNA. The rest of the density, which must be coat protein, can be roughly divided into four approximately radial ribbons between 40 and 70 Å radius, two columns of density at 22 and 32 Å and an island of density between 75 and 85 Å. We can use the chemical information available about the protein to trace some of the chain.

Three of the ribbons form one continuous chain of density touching the RNA at two points. Residues 27 and 68 are labelled by heavy atoms, and appear to be in this chain. Since we believe the N-terminus to be at a radius of about 90 Å (see Table 2), we continue the chain back from residue 27 into the island, crossing at the only point which avoids very low densities.

Residue 139 is labelled, and is close to the inner edge of the island, so it seems that the C-terminus is also at high radius. This is confirmed by its availability to carboxypeptidase[20]. Therefore, the fourth ribbon must take the sequence from the RNA interface back to the island, and all the density inside the RNA helix must connect the third and fourth ribbons. This would agree with Butler and Durham's[18] location of the lead on residues 115 and 116.

The inner density is difficult to follow, but a tentative interpretation can be made. It seems that the chain probably runs up the inner column and down the outer, before crossing the RNA to join the fourth ribbon.

An interesting feature of this interpretation is that the island contains the C-terminus of one subunit and the N-terminus of the adjacent subunit. We would emphasise that this feature of the interpretation is somewhat preliminary.

Figure 4 consists of two photographs of balsa wood models of three subunits of TMV protein and strands of RNA. It is clear that the structure is essentially four fairly straight chains, with the RNA in a hollow on top of the molecule.

Conclusions from the map

Even though the map is only at 6.7 Å resolution, the following conclusions may be drawn:

First, the map suggests there is little β sheet since this would show up as non-resolved regions of high density at this resolution. Apart from very short sequences, the only place where a β sheet could be accommodated would be the island, which contains at most 20% of the protein.

Second, there are several regions of high protein density which would be consistent with α-helix, including two regions of high, rod-like density which are very good candidates for helices. These are probably in the vicinity of residues 55 and 95. α-helix was predicted at the first of these residues by Schiffer and Edmundson[21] and by Leberman[22], and Leberman also predicted the second. In fact, all of Leberman's five α-helical regions and five of Schiffer and Edmundson's six regions could be in or near our regions of high density.

Another interesting feature is the points of contact with the RNA. There are three, all in regions which are conserved in many strains and viable mutants of TMV. Furthermore, all three contain invariant arginines (residues 41, 90 and 92, 113) which have often been suggested as possible residues for interacting with phosphate groups.

Finally, the island of density is interesting. It seems to be joined to the rest of the protein by a region of low density which may be a hinge allowing some freedom of movement in the *z*-direction. Caspar and Holmes[23] concluded from studies of the *Dahlemense* strain that the outermost part of the subunit in *Dahlemense* TMV is periodically perturbed. It is possible that the structure involved in the *Dahlemense* perturbation is the outer island and its associated hinge.

This work is continuing, although the maximum resolution obtainable from this approach is probably only 5 Å. Klug and his coworkers in Cambridge are working on the crystal structure of the two-layer 17-fold disks of the coat protein[24-26] and it should be possible to take this work to atomic resolution. Eventually we hope to combine the two maps to obtain an atomic model of the entire virus.

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Control of somite number during morphogenesis of a vertebrate, *Xenopus laevis*

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During Xenopus *embryogenesis and early growth, somite number stays close to a species-typical value for each morphological stage. This remains true even after operations on blastulae which lead to the development of abnormally small but otherwise complete early embryos, involving reduction in number of cells assigned to each somite. Evidence presented suggests that a body-position gradient may be involved, but in rather different ways at different stages, in controlling total somite number.*

SPATIAL patterns of cell differentiation underlying morphogenesis in early embryos fall into two categories. One is the specification of non-repeating anatomical features such as heart, kidney, and limb and tail rudiments, henceforth referred to as the 'whole-body pattern'. The other is the specification of serially repeating units of morphogenesis, typified in vertebrate embryos by the somites, the segmental blocks of mesoderm from which serially repeated elements of axial skeleton and musculature develop. Development of whole-body and repeated patterns is coordinated in space, in that somite no. *n* in a particular species is quite reliably opposite, for instance, the hindlimb bud.

An elaboration of earlier gradient models, proposed by Wolpert[1], postulates a continuously graded cellular 'positional information' variable across tissue. Commitment of a cell to a given developmental pathway depends on its 'interpretation' of the value of the variable at a given point in the gradient. On such a model, although the spatial variable underlying pattern formation is simply distributed, the burden on the genetic machinery underlying cellular interpretation is correspondingly demanding, and the mechanism becomes implausible for serially repeated structures. This is because it is hard to see how the interpretative system within cells could be so organised as to react in a particular way, for example by deadhesion to form fissures between the successive, similar somite blocks, only to some thirty, regularly dispersed values on a gradient, although ignoring or reacting differently to intervening ones.

Models that postulate prepatterns[2-5] in developing tissue have therefore been preferred. These are defined as the regular repetition in space of some special situation (for example a concentration peak of a 'morphogen' substance), each repetition causing development of one of the pattern elements subsequently seen in differentiation. The problem of interpretation by cells then becomes simplified, perhaps even to a binary choice controlled by a threshold concentration (for example, deadhesion as opposed to adhesion for somites, bristle-cell as opposed to epidermis for certain insect patterns). There are, however, other difficulties, connected with repeatability and consistency of the patterns in

space. In the case of somites, the observed constancy and regularity of element size and number[6] is embarrassing for all known prepattern models[7,8].

Regulation is an even more profound problem. When overall cell number of early vertebrate embryos is reduced, cell numbers developing along each pathway are reduced to give a normally proportioned whole-body pattern. There are several plausible mechanisms for regulating the steepness of a continuous gradient to account for such regulations[1]. The distance between 'peaks' in prepatterns underlying repeated structure, on current models[3,4], is determined, however, by physiochemical parameters (diffusion and allosteric interaction constants for specific molecules) which cannot easily be imagined as modifiable by changing the overall dimensions of the tissue. A prediction is therefore that the number of elements in a repeating pattern will be reduced from normal in direct proportion to amount of early embryonic tissue removed, if the latter amount corresponds to more than one whole unit's worth.
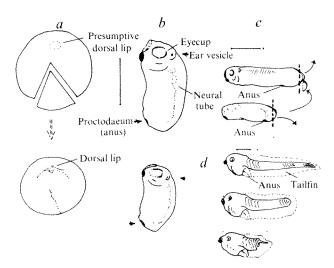
Fig. 1 *a*, Vegetal view of stage 8 blastula showing size reduction operation. This was performed in two-thirds strength Niu–Twitty solution[13], but with the $Ca^{2+}Mg^{2+}$ (Cl⁻) concentration doubled and *p*H brought to 7.0 with HCl. Healing was for 1 h in a fitting depression in wax, before storage to develop in one-tenth strength solution. Healed small early gastrula shown. In control siblings only one of the two cuts used to remove material was made, and healing allowed in the same solution. Normal repositioning of an excised section could not be achieved, and development of such animals was too disturbed for use as control material. *b*, Neurulae at stage 25, control and experimental, showing a size difference close to the maximum compatible with final development of experimental to the later larval stages. *c*, Early 40s stage larvae, control, and after tailbud tips removal at stage 24 and stage 26. Scale line represents approximately 1 mm throughout.
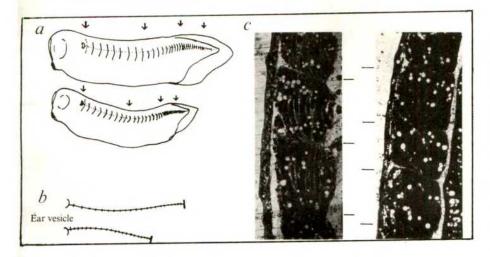
Fig. 2 *a, Camera lucida* drawing of an experimental and control pair at stage 34/35, each having 35 or 36 postotic somites, as seen after epidermis removal during fixation. Postotic axial length ratio now 0.83, having been 0.70 in stage 23 tailbud embryos. Arrows mark ear vesicle, 10th, 20th and 30th somites, seen to be appropriately placed. Somite boundaries are marked, but their outlines not drawn. *b, Camera lucida* record of somite boundaries in isolated axes between ear vesicle and level of proctodaeum. Length ratio best estimate is 0.75. Experimental has 21–22 somites, whilst its control has 23. *c,* Horizontal 1-μm epon sections stained with toluidine blue, showing mid-trunk somites of an experimental and its control at stage 25/26. The shortness of the experimental somites can be seen. Marker lines are opposite inter-somite boundaries.

Regulations of somite number

Measurements of total length and of total somite number, in synchronously developing sibling *Xenopus* larvae, show no detectable correlation between the two. This suggests that despite variation in amount of egg material, total length variation in the somite-forming tissue is not an important source of variation in somite number. I have tested this conclusion further by removing animal-vegetal sectors from near the ventral meridian of stage 8 (ref. 9) blastulae (Fig. 1a and b) following which tailbud stage embryos are obtained of as little as five-eighths normal length. These animals have developed a complete whole-body pattern across their reduced number of cells. Cell counts per area in epidermal preparations show no evidence that extra mitoses have occurred by these stages in the experimental animals; they have regulated in the classic sense that the positional information theory[1] was evolved to explain.

I have counted and measured the numbers and sizes of post-ear-vesicle somites in operated embryos and their control siblings, fixed at the same time post fertilisation. Embryos down to little over two-thirds control length show essentially normal numbers and relative positions of somites at each stage (Fig. 2 and Table 1), revealing that the available prepattern models cannot account for somite formation.

Another class of mechanisms which can be clearly ruled out are those that would depend on particular, species-specific cell numbers. The length of individual somites seems to be determined by total length of tissue available, and numbers of cells per somite adjusted accordingly. Both within and between control embryos, cell numbers in the original lengths of somites are almost constant throughout the trunk region, but in haploid embryos, having more, smaller cells than normal, cell number per somite is correspondingly larger[6]. This is easy to check because true mitosis in *Xenopus* somites is very rare after the process of deadhesion and rotation of successive blocks of somite cells[6]. Cell numbers across somite widths in later embryos are thus records of the numbers between fissures at the time of somite formation. In my experiments, the smaller somites in fact contain fewer cells than normal in this dimension, which is the one determining how many somites are 'fitted in' to the whole-body pattern.

Still smaller embryos tend not to develop beyond mid-tailbud stages, and to have poorly defined somites that cannot be counted. This may be for mechanical reasons such as reduced stretching of the normal-sized cells, so that they are insufficiently spindle-shaped to perform rotation movements, but other explanations to do with the as yet obscure process of pattern formation itself are possible. One exceptional embryo, measuring only 0.6 of control length between ear vesicle and rear end, showed 4 clear rotated somites on

Table 1 Comparison of numbers and lengths of somites formed behind ear vesicle level, relative to length of axis traversed by the somite morphogenetic process, in experimental small embryos and synchronous control siblings

Sets of synchronous siblings operated at stage 8	Total mesodermal length behind ear vesicle as % of control length	Proportion (%) of distance from ear-vesicle–proctodaeum (anus) occupied by somites	No. of rotated somites posterior to ear vesicle	Thus, total length of mesoderm behind ear vesicle incorporated into somites, as % of control value	Length of equivalent set of 8 (3–10 post ear vesicle) somites (%)	No. of cells in width (original length) of some anterior trunk somites, averaged from counts on 4 separate sections per somite
Set I Controls 1	—	69	11–12	—	—	
(stage 24+) 2	—	74	11–12	—	—	
Experimentals 1	77	73	11–12	79	78	
2	76	75	11–12	77	78	
3	65	74	10–11	68	76	
Set II Control	—	90	17–18	—	—	
(stage 27) Experimental	80	90	16–17	79	81	9.5 10.0 9.5 9.75 10.0 10.5 10.25
Set III Control	—	100 (opp. anus)	20	—	—	9.0 8.5 8.5 8.75 8.0 8.25 8.5
(stage 29) Experimental	80	over 95	18	74	79	
Set IV Control	—	100 (opp. anus)	21	—	—	
(stage 29) Experimental	68	85–90	16	61	65	
		% of total post e.v. mesoderm occupied by somites		Thus, total length of somitised mesoderm as % control		
Set V Control	—	73	22–23	—	—	
(stage 31) Experimental	85	76	20–21	89	72	9.75 9.5 10.25 9.75 10.25 10.0 9.5
Set VI Control	—	85	28	—	—	7.5 7.0 8.0 7.5 7.75 8.0 6.75
(stage 32) Experimental	90	88	26	92	81	
Set VII Control	—	about 95	33+	—	—	
(stage 34) Experimental	86	about 95	33+	86	84	

Note: "Somite morphogenesis extends to tailbud" appears at the left between Set IV and Set V rows.

Embryos were either prepared for wax or epon sectioning, or stripped immediately of epidermis for somite counting and measurement. Measurements were derived from constant scale *camera lucida* drawing, and cells in widths of mid-trunk somites were counted at notochord level in horizontal section[6]. Nuclear counts in epidermal whole mounts and sections give no evidence of smaller cells, as a result of compensatory mitosis since operations, by early tailbud stages. Sets consist each of experimental and synchronous control embryos, fixed at the same time after fertilisation.
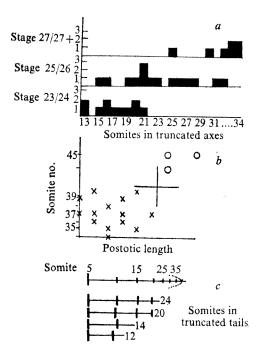
Fig. 3 *a*, Histogram of somite numbers in truncated axes of a population of siblings at stage 39, following tailbud tip removal at stages 23/24, 25/26 and 27/27+, respectively. Line represents average postotic somite number in synchronous control (unoperated) population. *b*, Scale chart of mean *camera lucida* positions of boundaries between successive groups of 5 postotic somites in the control population of *a*, in comparison with records, drawn in alignment, for four individual truncated axes.

one side, anteriorly, each 5–6 rather than the normal 9–10 cells in width (original length).

Yet another alternative model, in which appropriately proportioned blocks of cells are cut off by a local adaptation of the lengths of blocks to the width and depth of the column of presomite cells (the latter dimension regulating as part of the whole-body pattern after operations), can be ruled out. In the related amphibian *Bombina*, the morphogenesis of which is slower, I have been able to remove, at the close of gastrulation, a longitudinal strip of the sheet of cells that will later form the somite column in the trunk region, on one side only. Such animals develop with the column of presomite cells much shallower and narrower on the operated than on the normal side, over a distance equivalent to many somites. Somite numbers formed, however, prove to be equal on each side, in register, and normal for the species.

Somite number and growth controlled together during tail development

During mid-tailbud stages in amphibian embryos, when the above observations were made, the first true cellular growth, as opposed to cleavage, occurs especially in the tailbud region. Somite segmentation extends progressively into this, posteriorly, with rapid growth occurring at all levels not yet reached by onset of notochord and somite histogenesis. But increasing somite number remains in register with morphogenesis throughout growth in both control and experimental (initially small) animals, so segmentation must continuously be adapted to total length of tissue available.

The problem of coordination of growth and differentiation arises most acutely for structures such as limb and tail rudiments, in which the two processes are proceeding simultaneously. Summerbell *et al.*[10] have suggested that in the developing chick limb, positional value of mesoderm cells, though they are dividing throughout the organ in true growth, changes with time only in a terminal un-

differentiated 'progress zone', where progressively more 'distal' values are achieved. Thus following successively later apical ectodermal ridge removals from wingbuds, a succession of more nearly complete, yet still distally truncated patterns were finally differentiated.

I have performed analogous experiments on *Xenopus* early tailbuds, and the results suggest that pattern formation is controlled in a similar manner. Following a suggestion of Graeme Mitchison, a terminal piece of undifferentiated tailtip mesoderm with ectoderm, always some 300 μm long, has been removed from sibling embryos at a continuous range of tailbud stages, followed by counting of the total somites formed in the truncated tails of the advanced larvae 48 h later. Figure 3 shows typical results, from one of several such experiments consisting of 33 tailtip removals and 14 controls. Following tip removals between stages 23 and 27, that is, well before somite formation has reached the undifferentiated tip issue, the tails of the advanced larvae are sharply truncated, ending in short cone- or rod-shaped blastemas. They contain appropriate numbers of normal sized somites up to the levels of truncation (as measured by length), and the tailfin morphology, cumulative tail growth and lengths of successive groups of five somites are all literally parallel with controls up to the truncation point (see Figs 1c and 3c). The correlation between the proportion of a whole axis remaining, as judged by these criteria, and age of tip removal (Fig. 3a) is highly significant in spite of inevitable variation in the amount of tissue removed, particularly from very squat early tailbuds.

From Fig. 3a it seems likely that the somite numbers actually seen in truncated tails are a random sample from all possible integer numbers up to that in controls. When all the experimental populations are considered, no gaps in the series or traces of any stepwise distribution are seen. Taken together, these results indicate that position value of cells is fixed, as a continuous variable[5] of some nature rather than in prepattern terms, very early during tailbud growth, and probably when individual cells are near the tip. Thereafter, it would be maintained with at most a local 'averaging' between neighbour (daughter?) cells.

Alternatively, the position values for an entire tail (that is for all the somite complement) could be present across the cells of a very young, squat tailbud, and thereafter maintained by clonal inheritance and averaging throughout growth. Then, removal of a uniform amount of tailtip from progressively older tailbuds would result in truncation of progressively less of the distal portion of a position gradient, and hence of somite pattern. This concept would however imply one of two novel features: either an initial gradient, at a time of setting up, that is much steeper posteriorly than in the trunk (implied by the large number of presumptive somite positions there in relatively little tissue) or else a changing relationship, posteriorly, between 'wavelength' of the somite forming process and gradient steepness. The former is possibly not readily allowed for by any of the models for maintaining gradients[1], but the second idea cannot be discounted at present. Indeed, the number of cells in the width (that is the length, on first formation) of somites does decrease in a regular way posteriorly (my unpublished data).

Idea of a locally inherited position value gradient

Amphibian mesodermal cells are probably undetermined with respect to whole-body position value (however this is encoded) only in two situations: while within the regulating positional information gradient that embraces trunk values and exists across the early embryo until around gastrulation (see the miniature whole embryo discussed earlier), or else while in a 'progress zone' of labile region near the tailbud tip, where the graded series of values is extended to complete the axis. Such a position gradient, if preserved by

local mechanisms among descendants of cells, would still be available to cause coordinated placement of later-developing structures (for example: limbbuds, or the special behaviours of particular spinal nerves in the series[11]).

The results discussed indicate that the steepness, or rate of change within embryonic tissue, of the positional variable is able to control the distance between repetitions of a certain cellular event in the animal's long axis. For reasons given at the outset, it is hard to imagine that this could be by direct interpretations of positional values as has been postulated for the whole-body pattern, but a theory of somite control, accounting for the known features of the process and its coordination with other morphogenesis, is to be presented elsewhere[12].

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Positional signalling and specification of digits in chick limb morphogenesis

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The interpretation of positional information can provide the basis for pattern formation in limb morphogenesis. The gradient in positional information along the antero–posterior axis, which is specified with respect to a localised boundary region, can be modified by grafting this region to successive positions along the axis. The pattern of digits obtained is consistent with a model based on diffusion of a labile morphogen and is thus similar to models proposed for the development of pattern in invertebrates.

RECENT approaches to pattern formation, that is the development of the spatial pattern of cellular differentiation, have made use of the concept of positional information[1]. The suggestion is that cells are assigned positional information in relation to a coordinate system and the interpretation of this leads to appropriate cytodifferentiation. This is essentially an extension of earlier gradient models[2]. Good evidence for positional information comes mainly from studies on the insect epidermis[3,4], insect eggs[5] and regeneration in hydra[6,7] but there are few demonstrated instances in vertebrates. We have suggested that the development of the pattern of the cartilaginous elements of the chick wing can be viewed in terms of the concept of positional information[8,9]. The positional values assigned to the cells define a set of cell states, some of which will, for example, result in cartilage formation and others in the formation of muscle. We have proposed that the cells are assigned positional values along two of the axes, the proximo–distal and antero–posterior, by somewhat different mechanisms, the two positional values providing a two-dimensional coordinate system (Fig. 1). For the proximal–distal axis we have presented evidence that there is autonomous change of positional value with time in the progress zone[8,10]. This is a region about 300 μm thick at the distal end which is specified by the apical ectodermal ridge (AER). Since all the cells in the progress zone are dividing, cells constantly leave the zone, those coming out later being assigned more distal positional values. This mechanism does not require signalling between the mesenchymal cells, although there may well be short range interactions, or local interactions involving averaging

of positional value. By contrast, for the antero–posterior axis, we suggested that the positional value is specified by a positional signal from the polarising region at the posterior edge of the progress zone[9,11]. This was a conjecture based upon the work of Saunders[12,13] and we now present evidence for a signal from the polarising region providing positional information for the specification of the digits in the chick wing. Crick[14] has emphasised that diffusion could provide the basis for positional information and our data is consistent with such a mechanism.

The zone of polarising activity (ZPA) was discovered by Saunders and Gasseling[12]. An additional ZPA grafted to a more anterior level in contact with the AER, resulted in the formation of extra skeletal elements. Of particular interest was the relationship of the additional elements with the normal ones; when between the host ZPA and the grafted ZPA, they were always in mirror image symmetry about the long axis. Moreover the polarity—the order of the induced digits—was such that there was a strong
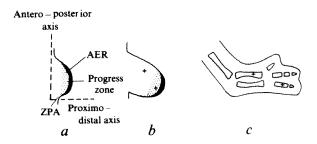
Fig. 1 The chick wing bud starts as a small bulge (*a*) and grows out as a tongue-shaped mass consisting of mesenchyme encased in ectoderm. The ectoderm has a thickening running along the distal rim, the apical ectodermal ridge (AER). The skeletal and muscular elements differentiate within the mesenchyme in a proximo–distal sequence (*c*). It is suggested that cells are assigned positional information when they are in the progress zone, along, for example, the antero–posterior and the proximo–distal axes. In the case of the antero–posterior axis, position is specified with respect to distance from the zone of polarising activity (ZPA). In *b* there are two points marked (+), the one outside the progress zone has already had its positional information specified, while the other is in the process of being specified. At a later stage, cells at these points interpret the positional information giving rise to tissues of the radius and digit 4 respectively (*c*).

Somite Number	Predicted effect of ZPA grafts at different levels
14/15	z
15	z
15/16	z
16	z
16/17	2 3 4 z 4 3 2
17	2 3 4 z 4 3 2
17/18	2 3 4 z 4 3 2
18	2 2 2 2 2 2 3 4 z 4 3
18/19	3 3 3 3 3 3 3 3 4 z 4
19	4 4 4 4 4 4 4 4 4 4 z
19/20	z z z z z z z z z z z
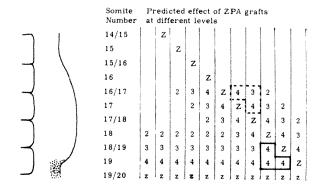
Fig. 2 At the left is the outline of the normal limb bud and the somites at stage 19: the normal position of the ZPA (z) is also shown stippled. The predicted pattern of digits when an additional ZPA (z) is grafted to successive positions along the antero–posterior axis is shown in the columns on the right. It is based on the assumption that the digits are specified with respect to their distance to the nearest ZPA, that is a simple linear gradient. The predictions are only meant to show in diagrammatic form the overall pattern, and changes in limb geometry have not been allowed for. Nevertheless all the cases have been found experimentally except those enclosed by the solid line where either no digits were formed, or were much reduced. Those cases in which the digits are enclosed by a dashed line, are where digits formed but were sometimes inconsistent with the prediction, that is instead of 43, either 432 or 32 was obtained.

tendency for posterior structures to be adjacent to the graft[13]. We have interpreted these results as implying that the ZPA is the boundary or reference region for the antero–posterior axis of positional information[9,11]. Since only cells in the progress zone can respond to the influence of the ZPA[15] the character of the digits may be specified by their distance from the ZPA at the time when they are leaving the progress zone. One way in which a gradient in positional value could be set up would be by the ZPA being the source of a diffusible morphogen which would act as a positional signal.

In the first instance we will consider a model which is not dependent on any specific assumptions with respect to mechanism, such as diffusion, and only assumes that positional information is specified by the distance from the

Table 1 Formation of digits between the grafted ZPA and the host ZPA when the ZPA is placed in successive positions along the antero–posterior axis

Stage 18 Digits	Position of graft with respect to somites									
	14/15	15	15/16	16	16/17	17	17/18	18	18/19	19	19/20
234		6									
2234		1									
32234		1	3								
432234			4	3							
43234			3	5							
4334					4	5	2	1			
434						1	4	4	1		
44											
4								1	2		
None									2	4	
Stage 20											
234	7	3	2	2							
2234		1	2		1						
32234		1		1							
432234		1	2	2	1						
43234		1		3	2						
4334					3	4*					
434						1	5*				
44											
4								2			
None								2	8	11	2	1

The data refer to the number of cases obtained.
* In some of these cases the identification of the anterior digit 4 was equivocal.

nearest ZPA; that is we assume a simple linear gradient. The best markers along the antero–posterior axis are the three digits (II), (III) and (IV) (which for convenience we will represent as 2, 3, 4) since they can usually be recognised unequivocally by the number of elements and distinct morphological characteristics. We will thus confine our attention to them and assume that the nature of the digit specified is determined by its distance from the nearest ZPA. We have shown diagrammatically the predicted pattern of digits when an additional ZPA is placed in successive positions along the antero–posterior axis, the host's ZPA being left intact (Fig. 2). From this several interesting features emerge. First, digit 2 will form wherever the gradient has an appropriately low level and thus it should be possible to obtain an additional digit 2 on its own when the ZPA is placed in a sufficiently anterior position. Second, one does not get discontinuities such as digit 2 next to digit 4. Third, there is no requirement for intrinsic polarity, that is the signal from the ZPA propagates in both directions.

In the normal limb the digits form mainly in the posterior half of the limb, that is from the region between somites
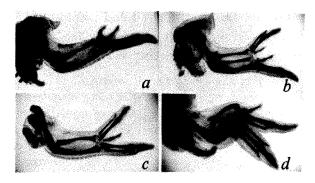
Fig. 3 A series of dorsal views of whole mounts of right limbs with grafts showing the pattern of digits formed as the grafted ZPA is placed in successively posterior positions. The digits formed are as follows from anterior to posterior; *a*, 2234; *b*, 432234; *c*, 43234 and *d*, 234434.

18 and 19[16]. The ZPA occupies a position immediately posterior to this (Fig. 2). The anterior half of the limb does not seem to participate in formation of distal structures and this may be related to the asymmetry of the AER which is better developed posteriorly.

Grafting the ZPA

We have substantially confirmed all the predictions by grafting ZPA tissue to successive positions along the antero–posterior axis. ZPAs were taken from stages 18 to 21 and pinned with a small piece of platinum wire into a hole cut into the edge of the limb[15]. The graft was thus always in contact with the AER except when placed in more anterior positions. We have made use of the somites as markers for position along the antero–posterior axis. Since the distance between somite centres is about 250 μm and the size of the ZPA graft is about the same, the error in the positioning of the graft is about half the width of a somite. The results are substantially the same for stage 18 and 20 hosts (Table 1).

Considering structures posterior to the graft first, it can be seen that when the graft was opposite somite 15 which appears to be just outside the anterior border of the limb bud, normal limbs developed in most cases. This confirms that the ZPA from the wing does not itself differentiate into any recognisable structure[15]. This suggests that the signal from the ZPA is rather short range and that body wall tissue is not competent to respond to the signal. Of particular importance is the development of hands comprising 2234 (Fig. 3a) and 32234 when the graft is in the
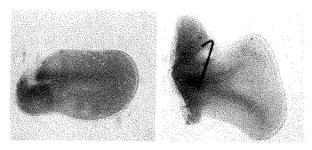
Fig. 4 Dorsal view of whole mounts of the normal and grafted right limbs at stage 24. The ZPA was placed opposite somite 16 at stage 18 and the width of the limb has increased by 50%. Note the pin which held in the grafted ZPA.

region of somites 15 and 16. This shows that the ZPA can specify either digit 2 or digits 23 without formation of digit 4, and thus precludes any mechanism for specification of the digits based on sequential induction. On the contrary this result is consistent with a graded signal which specifies digit 2 when the lower part of the gradient first begins to lie within competent limb tissue. It is of interest that this result also suggests that tissues can transmit the signal without responding to it. Similarly, in a variety of other grafts where we had reason to believe that the signal from the ZPA has been attenuated by, for example, the presence of a Nucleopore or Millipore filter, it is often digit 2 only that is specified.

As the graft is placed in successively posterior positions, digit 4 develops anteriorly and a complete mirror image duplication occurs (Fig. 3b); and when placed still more posteriorly, first one digit 2 and then the other, is no longer formed between the two grafts (Fig. 3c). When the graft is opposite somites 17 to 18 only a 4334 or 434 forms posterior to the graft. When the graft is, however, moved slightly more to the posterior, contrary to expectation, no 44 combinations could ever be identified with certainty. On occasion, a small single 4 could be found. When the graft was placed opposite somites 18 and 19 at stage 18, which is quite close to the host's ZPA, normal limbs were usually formed, the digits being anterior to the graft. This suggests that the signal from the ZPA is not affected when two ZPAs are placed together. But, at stage 20 this graft often resulted in the complete absence of digit 4.

The structures formed anterior to the graft were more variable. Nevertheless, they conform quite well to the predictions of Fig. 2. Digits anterior to the graft usually only formed when the ZPA was placed opposite somite 17 or more posterior. The most common results observed were 23 and 234. At stage 18 some grafts resulted in 34 and single digits, possibly digit 4. Where a complete anterior hand 234, was present, the posterior hand was always 434 or less (Fig. 3d). One exceptional case produced 2344334, the largest number of digits we ever obtained along the same antero–posterior axis.

The results as a whole conform well with both the general and specific predictions of Fig. 2. The main deviations occurred when a single or double digit 4 were predicted in isolation. In this connection we have some evidence that a digit 4 begins to develop but fails to do so because of degeneration of the associated vascular system.

As Saunders[15] has pointed out, the grafted ZPA can bring about changes in the extent of the AER and thus the outgrowth of the limb. In fact, placing the ZPA in a variety of positions near the tip of the limb is without effect unless it is adjacent to the AER. In the normal limb the outgrowth is not symmetrical about the long axis (Fig. 4). By contrast when a ZPA is placed anteriorly (level somite 16) the outgrowth is symmetrical about the long axis, the limb increasing in width. Measurements of the change in width in operated limbs show that the increase in width seems to

correlate with the increase in number of digits obtained. From the point of view of digit specification the crucial width is that at stage 24 when the digits begin to be laid down[17]. For example, Fig. 4 shows the difference at stage 24 between a normal limb and one which had a ZPA grafted opposite somite 16. There is a 50% increase in width. It should be noted that there does seem to be a constraint on the number of digits than can be specified: this is very rarely greater than six.

Diffusion model for the signal

The predictions of Fig. 2 are based on a simple linear gradient and the question now arises whether this gradient could be provided by a diffusible morphogen. In general terms, diffusion would seem to provide a suitable mechanism particularly since the signal from the ZPA is propagated in both directions, and no discontinuities in level were found. Moreover the time/distance relationships are compatible with diffusion since the gradient is set up over about 500–1,000 μm and the time available is of the order of 10 h. It is perfectly reasonable to set up a diffusion gradient over this distance in the available time[14].

It is possible to set a linear diffusion gradient with a source and a sink at the ends[14] but in the chick wing, although the ZPA can be considered as a source, there does not seem to be any region corresponding to a localised sink. Thus it is necessary to assume the morphogen to be broken down and this will give a gradient with an exponential form. In this case the concentration of the morphogen at a given distance from the ZPA will not be constant, but will vary according to the presence of the second ZPA and this is inconsistent with Fig. 1. Ignoring the complex geometry of the limb, consider the simple one-dimensional case in which the ZPA would be the source of a morphogen which is broken down; the concentration at the ZPA being fixed (Fig. 5). If another source is now placed at the other end, analogous to placing another ZPA at the anterior border of the limb, the resulting concentration of the morphogen is as shown in Fig. 5, the concentration in the central region being considerably increased. Such a distribution is inconsistent with the results, for on any

Distance (mm)
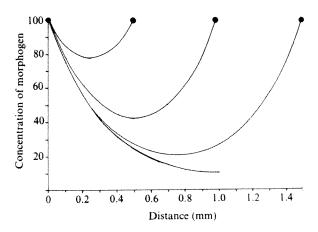
Fig. 5 The concentration profile of a morphogen for the one dimensional case, which is taken to represent the antero–posterior axis. The source (●) of the morphogen, representing a ZPA, is fixed at 100 and the diffusion constant and rate of breakdown were chosen such that, for the case of a single source, the value 1mm away from the source would be 10. The diagram also shows the profiles of the morphogen when a second source is added at varying distances along the axis. The concentration in the middle region is that most affected and is no longer that corresponding to the case of the single source. For example, when the two sources are 1mm apart, the region 0.5 mm from the ends has its concentration increased from 24 to 42. If the distance between sources is increased to 1.5 mm, however, the concentration 0.5 mm from the end is relatively unaffected.

plausible values of the concentration for specifying the digits, a graft opposite somite 16 should result in the absence of digit 2 in the middle region which is not the observed finding. But we have so far ignored the fact that when a ZPA is grafted to an anterior position the width of the limb bud does not remain constant, but increases substantially. If there is an increase in width of the distal region of the limb, the profile of the morphogen will be significantly altered (Fig. 5) since the sources are now further apart. For the highly idealised case, an increase of 50% would give a profile compatible with the results. More complex models, taking into account a homeostatic component[3] would also tend to reduce the concentration in the central region, but if the increase in width had not occurred it would have made a diffusion model very unlikely. There is in fact some evidence that cells maintain their positional value when the signal is removed, for the total removal of the ZPA and the region that will normally give rise to digit 4 leads to the loss of digit 4, but digits 2 and 3 are normal.

Our results strongly suggest that the digits in the chick wing are specified by a gradient in positional information, the nature of the digit being determined by its distance from the ZPA. The results are consistent with the specification of positional value by diffusion of a labile morphogen from the ZPA, which may be considered as a boundary region. This implies that the character of the digit is specified by a concentration gradient acting as a positional signal, but we do not know how this is read off by the cells. It should be noted that we have assumed specification of the digits to occur at the same time: it is possible to have a model in which they are specified by a series of sequential decisions (J. Lewis, personal communication). The ZPA also seems to affect the outgrowth of the limb, the width being significantly increased. This may be mediated by the

AER and the ZPA has little effect if not in contact with it.

Although we have concentrated only on digit specification here this gradient would provide the positional information along the antero–posterior axis for all elements of the limb and it is clear that the ZPA does have an effect on the proximal elements such as humerus, radius and ulna[11,14]. Moreover, it is known that a similar system is operative in the leg and the ZPA of wing and leg are interchangeable[13]. It thus shows that in the chick limb, pattern formation can occur in a way strikingly similar to that found in some invertebrates, and strengthens the view that there may be universal mechanisms. Similarities to the classical organiser may also be noted.

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letters to nature

Local interstellar medium

NEUTRAL interstellar hydrogen can be prevented from penetrating freely into the heliosphere of solar plasma, because it is partially coupled to the subsonic flow of interstellar plasma. Results from modelling the backscattering of the diffuse Lyman-α radiation are therefore unreliable. The interstellar density of H$^+$ is probably 0.02 cm^{-3} or less, and the density of neutral hydrogen greater than 0.2 cm^{-3}.

Present thinking favours the idea that the interstellar medium in the neighbourhood of the Solar System is low density "intercloud" gas. With heating by postulated MeV cosmic rays or soft X rays, a stable state could exist[1] with atomic hydrogen density $N \simeq 0.1$ cm^{-3}, temperature $T \simeq 8,000$ K and an ionised component of H$^+$ and e$^-$ of density $n \simeq 0.03$ cm^{-3}. The plasma density might be larger because of the ionising ultraviolet emission from local hot stars[2].

Observations of Lyman-α absorption in the spectra of nearby stars indicate[3,4] generally higher values of the mean gas density $N \simeq 0.2$–0.6 cm^{-3}. The interpretation of the all-sky maps of the diffuse Lyman-α backscatter is still the subject of debate: the various models give[5-7] densities $N_L \simeq 0.05$–0.25 cm^{-3}, and streaming velocity relative to the Solar System $V = 10$–20 km s^{-1}, but are inconclusive about the temperature. Both sets of data imply that the local medium would be the low density, intercloud state, being far from the typical density 10 cm^{-3} of

clouds[1]. But if, as I argue, the density N_L within the Solar System is significantly lower than the undisturbed gas density N it seems that the local medium is not particularly close to the theoretical intercloud state.

My argument is based on the global picture of gas and plasma flow in the neighbourhood of the heliosphere (Fig. 1). Two plasma shocks are shown, one terminating the supersonic solar wind and the second a bow shock[8,9], while a contact surface separates the interstellar plasma from the heliospheric plasma originating from the Sun (which acts as a source). The bow shock is a fast hydromagnetic shock and exists with the plasma parameters given earlier only if the magnetic field is rather weak: for $B < 10^{-6}$ gauss, the magnetosonic Mach number $M = V/[(B^2/\mu_0 nm) + (2\gamma kT/m)]^{\frac{1}{2}}$ can exceed unity. The plasma behaves as a fluid on a small, ion gyro-radius scale while the neutral gas moves almost freely through it. But, in practice, the gas must interact with the plasma on the large heliosphere length scale, effecting frictional drag and cooling and so distorts the flow from the standard one of a supersonic source in a supersonic stream. Indeed, the terminating shock of the solar wind is probably weak or even non-existent[10] because the plasma–gas interactions effect deceleration over an extended region, analogous to the expansion of ordinary gas into a rarefied background medium[11]. The plasma–gas interactions dominate if $B/N_L \gtrsim 10^{-5}$ gauss cm^3, in which case the heliosphere radius is[10] $R \simeq 60 N_L^{-1}$ AU (where N_L is in cm^{-3}), about twice the sonic position's mean radial distance.
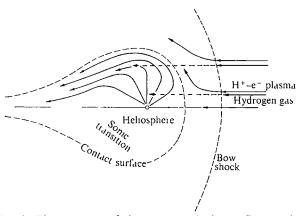
Fig 1 The structure of the magnetised plasma flow in the neighbourhood of the heliosphere The streaming hydrogen gas is partially coupled to the plasma, its direct component being depleted in penetrating the diverted and shocked interstellar plasma

If the plasma–gas interactions were negligible the bow shock would stand off ahead of the heliosphere by a distance Δ, given roughly by

$$\Delta/R \simeq 1\ 1n/n' \tag{1}$$

where n' is the density downstream of a normal plane shock

$$n'/n = (\gamma + 1)/(\gamma - 1 + 2M^{-2}) \tag{2}$$

Expression (1) is applicable[12] in a wide range of values of γ and M, and even for weak shocks (see section 4 4 and Fig 6 27 of ref 13) with n'/n as low as 1 1

The main plasma–gas interaction is the resonant H^+-H charge exchange process with a cross section at 1–3 eV ($\frac{1}{2}mV^2$ or kT) of $\sigma = 6 \times 10^{-15}$ cm² I can thus estimate the proportion of streaming hydrogen atoms which pass through the stagnation region into the heliosphere as $N_L/N = \exp(- \lambda)$, where

$$\lambda \simeq (1 + v)^{\frac{1}{2}} \Delta \sigma n' \simeq 1\ 1\ (1 + v)^{\frac{1}{2}} R \sigma n \simeq 8n/N_L \tag{3}$$

The factor involving v represents the increased collision rate in thermal plasma[10] and is adequately approximated by taking $v = 0\ 7-1\ 0$ in the present situation

With the values n and N_L given above, the incoming hydrogen gas is appreciably depleted, by a factor exceeding three, and

$$N \simeq N_L \exp(8n/N_L) \tag{4}$$

exceeds 0 65 cm⁻³

This estimate is of course, very crude Particles contributing to N_L can approach laterally, avoiding the highest density stagnation region, but then pass through a larger depth of subsonic plasma, also friction of the neutral gas causes expansion of the stagnation layer and increases Δ above the value (1), and a proportion of the neutralised protons penetrate into the heliosphere and contribute to N_L Additional losses caused by interaction with the heliospheric subsonic plasma have been ignored (this latter is much hotter[10] at O (100 eV), so is less dense by a factor 100 and has little effect) But these considerations are to some extent compensatory and do not seem important enough to change the estimate (3)

Another possibility is that the interstellar field B is stronger, so that the plasma flow is sub-Alfvenic and the bow shock absent It is then necessary to calculate

$$\lambda = \int [1 + v\ (s)]^{\frac{1}{2}} n'(s)\sigma(s) ds$$

through the upstream flow, with σ larger for the smaller relative velocities and with some outer cutoff The frictional drag of the gas would distort the flow more severely, but otherwise the result for λ should not be much different from equation (3) The principal change with a larger B would probably be a decreased heliosphere radius R, but by only a factor of two for a field as strong as 5×10^{-6} gauss (ref 10)

There was an early suggestion[14] that charge exchange interactions of shocked solar wind protons with hydrogen-atoms of interstellar origin could contribute to N_L and to the diffuse Lyman-α backscatter In a curious sense, I have here reached a contrary conclusion that charge exchange processes impede the penetration of gas particles through the interstellar plasma into the solar system and reduce the local density N_L The interstellar gas is indeed not only depleted, but its thermal and streaming velocities must also be changed through the substitution of some neutralised protons Thus, analysis of the Lyman-α backscatter can tell us little directly about the real state of the local interstellar gas With the crude correction (4), the real gas density is compatible with the Lyman-α absorption values[3,4] only if the plasma density is $i \simeq 0\ 01-0\ 02$ cm⁻³ It indicates that the local interstellar gas has somewhat higher neutral-hydrogen density and lower ion density than the standard theoretical values[1]

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Charged dust grains and excitation of rotational levels of interstellar molecular hydrogen

THE strengths of ultraviolet absorption lines of interstellar hydrogen have been measured in the spectra of several reddened early-type stars[1-3] Interstellar H_2 molecules are observed mainly in the two lowest rotational levels $J = 0$ ($para$-H_2) and $J = 1$ ($ortho$-H_2) with a ratio of populations corresponding to an average temperature $\sim 80\ K$ This temperature is consistent with the mean HI kinetic temperature determined in 21-cm absorption studies[4,5], whereas higher rotational levels up to $J = 5$ or 6 are populated corresponding to significantly higher excitation temperatures[3,6] in the range 150–200K These observations indicate that a process other than thermal gas collisions is responsible for populating the higher rotational levels The precise nature of the excitation mechanism is as yet unknown A process which could contribute to non-thermal excitation of rotational levels involves encounters of charged dust grains with interstellar hydrogen We have examined this mechanism and find that it could be of comparable, if not greater, importance in relation to other processes which have been proposed

Since most interstellar dust particles are believed to originate in the atmospheres of cool Carbon or Mira-type stars, the velocity of their injection into interstellar clouds is $\sim 10^8$ cm s⁻¹ (ref 7) The velocity spectrum of interstellar grains resulting from the slowing of such fast grains due to gas collisions in clouds is[7]

$$f(v)dv = A/v^2, \quad v_{max} \ge v \ge v_{min} \tag{1}$$

where A is a constant, $v_{max} \simeq 10^8$ cm s⁻¹ and $f(v)dv$ denotes the number density of grains in the velocity range v, $v + dv$ The

minimum velocity v_{min} is significantly greater than the thermal speed determined solely by thermal gas collisions Systematic dust–gas drift velocities are maintained in most regions of the Galaxy by the action of radiation pressure from integrated starlight[8,9] The total stellar radiation from the direction of the galactic bulge drives grains relative to gas at speeds $v \geqslant 10^5$ cm s^{-1} in a cloud with $n_H \simeq 10$ m^{-3} and $T \simeq 100$ K in the solar vicinity[9] In the following discussion we assume $v_{min} \simeq 10^5$ cm s^{-1} to be the minimum velocity of a typical interstellar grain The use of the distribution function (1) is strictly justified, if the slowing time of fast grains in the cloud is comparable to or greater than the residence time of grains within the cloud For a cloud of dimension ~ 3 pc, the dispersal time for grains with $v = v_{min} \simeq 10^5$ cm s^{-1} is $\sim 3 \times 10^6$ yr This is an order of magnitude greater than the characteristic stopping time of fast grains[7] Thus the concentration of suprathermal grains may have to be diluted by a factor ten below that of all grains But since charged grain acceleration mechanisms could operate in the interstellar medium the precise velocity distribution of grains remains uncertain and a larger proportion of grains may be suprathermal There is also the possibility that at least some of the clouds for which H_2 observations are available, lie in the vicinity of stellar sources of interstellar dust, so that a high concentration of suprathermal dust grains could exist In view of these uncertainties we tentatively adopt the simple distribution function (1) in the ensuing discussion The mean velocity of an interstellar grain is then

$$\langle v \rangle = \frac{\displaystyle\int_{v_{min}}^{v_{max}} vf(v)\mathrm{d}v}{\displaystyle\int_{v_{min}}^{v_{max}} f(v)\mathrm{d}v} = v_{min}\,[1 - v_{min}/v_{max}]^{-1} \ln[v_{max}/v_{min}] \quad (2)$$

$$\simeq 6\,9 \times 10^5 \text{ cm s}^{-1}$$

The role of the high energy tail of this velocity spectrum in contributing to the heating and ionisation of the interstellar medium has already been discussed[7,10] We confine our attention here to the behaviour of grains with velocities near to the estimated 'average" speed $\sim 6\,9 \times 10^5$ cm s^{-1} Such grains carry a net electric charge which corresponds to an electrostatic potential $\varphi \simeq 0\,1$ V–1 V (ref 11), and collisions of these charged grains with H_2 molecules are quasielastic with an effective cross section

$$\sigma = \pi(fa)^2 \quad (3)$$

where a is a grain radius and $f \geqslant 1$ Although the precise value of f in equation (3) is uncertain, it may be expected to exceed one by a significant factor This could arise from long-range electrostatic interaction of spherical particles as well as from the effect of surface irregularities and protrusions, such as whiskers, that may grow on grains which are in a state of rapid rotation On the basis of cross sectional data for charged radical–neutral atom interactions[12] and other considerations it seems reasonable to set $f^2 = 10$

The energy transfer to an H_2 molecule in an elastic encounter with a grain of velocity v is

$$\Delta E \simeq m_{H_2} v^2 \simeq 0\,021\,(v/10^5 \text{ cm s}^{-1})^2 \text{ eV} \quad (4)$$

where m_{H_2} is the mass of a hydrogen molecule Setting $v = \langle v \rangle = 6\,9 \times 10^5$ cm s^{-1} in equation (4) we obtain $\Delta E \simeq 1$ eV The excitation energy of the J^{th} rotational level is

$$E_J = 0\,0073\,J(J + 1) \text{ eV} \quad (5)$$

giving $E_J = 0\,31$ eV for the highest observed level $(J = 6)$ This energy is less than that transferred to an hydrogen molecule by a grain colliding with the "average" velocity given from equation (2) Charged dust grain–H_2 collisions must therefore

warrant serious consideration as a candidate for the excitation of rotational levels of interstellar H_2

The rate coefficients for the excitation of rotational levels by this process may be readily calculated For excitation of level, the rate coefficient is

$$R_J \simeq \pi f^2 a^2 \frac{\displaystyle\int_{v_J}^{v_{max}} vf(v)\mathrm{d}v}{\displaystyle\int_{v_{min}}^{v_{max}} f(v)\mathrm{d}v} [n_g/n_H]n_H \text{ s}^{-1} \quad ($$

where n_H, n_g are respectively the number densities of hydrogen and of grains, and v_J is defined by equating ΔE given by equation (4) to E_J given by equation (5) This procedure yields

$$R_J = \pi f^2 a^2 \xi\, [n_g/n_H]\, n_H \text{ s}^{-1} \quad ($$

with

$$\xi \simeq 7\,5 \times 10^5\,(1 - 0\,067\,\ln[J\,(J + 1)]) \text{ cm s}^{-1} \quad ($$

Assuming a mass fraction $\sim 0\,02$ of interstellar material in the cloud exists in the form of spherical suprathermal dust particles of density $s \simeq 1$ g cm^{-3} and radii a we have

$$0\,02 = (n_g\,4/3\,[\pi a^3 s])\,/(n_H\,m_H) \quad ($$

which may be re-expressed

$$\pi a^2\,(n_g/n_H) \simeq 2\,51 \times 10^{-21}\,(a/10^{-5}\text{ cm})^{-1} \text{ cm}^2$$

Using equations (7), (8) and (10) together with $f^2 \simeq 10$ obtain the results of Table 1

Table 1	Values of R_J*
J	$R_J\,(a/10^{-5}\text{ cm})\,/n_H$ (cm^3 s^{-1})
1	$1\,80 \times 10^{-14}$
2	$1\,66 \times 10^{-14}$
3	$1\,57 \times 10^{-14}$
4	$1\,51 \times 10^{-14}$
5	$1\,46 \times 10^{-14}$
6	$1\,42 \times 10^{-14}$

*Since all the rate coefficients vary as $\sim a^{-1}$, grains of radii 10^{-6} cm if they exist in comparable mass (as is suggested by ultraviol extinction data), would dominate the excitation processes in t present theory

For grains of radii $a = 10^{-5}$ cm the rate coefficients calculate here are of comparable if not greater magnitude than tho obtained for ultraviolet pumping (ref 13 and unpublished wo by S P T and P Joshi) for $n_H \geqslant 10$ cm^{-3} We also note th the excitation rate of the $J = 1$ level produced by the prese mechanism is greater than the para–ortho conversion ra produced by proton encounters[14] for a H$^+$/H ratio less tha 10^{-4}

In the preceding discussion it has been assumed (a) th most of the transferable energy in the encounter between an H molecule and a grain is converted into the rotational energ (b) that the excitation cross section is equal to the collision cross section, and (c) that the excitation cross section is ind pendent of the relative energy of the collision From classic considerations (a) and (b) are likely to hold true to a go approximation for quasielastic encounters between a "dumb-b type" diatomic molecule and a solid surface Experimental da on encounters between diatomic molecules and solid surfac support these statements[15] Assumption (c) is difficult to justi and could be valid only in a crude approximation Since t precise population distribution among the various rotation levels depends strongly on the validity of this assumption

more detailed quantum mechanical determination of the energy dependence of the excitation cross section is worthy of pursuit

The depopulation of levels with $J \geqslant 2$ occurs mainly by spontaneous emission, the rates of which are greater than 10^{-11} s^{-1} (ref 14) We note that this rate is much larger than the de-excitation rate of $1\ 73 \times 10^{-14}$ s^{-1} by grain collision for f^2 $t_H = 10$, $a = 10^{-5}$ cm and $\langle v \rangle = 6\ 9 \times 10^5$ cm s^{-1} Therefore, the population N_J of any level $J \geqslant 2$ may be assumed to be given by the excitation by collision of H_2 ($J = 0$) with grains followed by spontaneous decay The population of the $J = 1$ level may be governed only by grain collisions The values of N_J/N_0g_J so obtained using the spontaneous emission rates from Dalgarno and Wright[16] are given in Table 2 for different values of J

Table 2 Comparison of observed N_J/N_0g_J ratios with theoretical prediction

J	Observed N_J/N_0g_J			Theoretical (N_J/N_0g_J)
	ζ Oph	ε Per	ρ Pup	
0	1	1	1	1
1	$6\ 32 \times 10^{-2}$	$1\ 12 \times 10^{-1}$	$7\ 07 \times 10^{-1}$	$1\ 16 \times 10^{-1}$
2	$2\ 52 \times 10^{-3}$	$3\ 97 \times 10^{-4}$	$7\ 94 \times 10^{-1}$	$1\ 13 \times 10^{-4}$
3	$2\ 24 \times 10^{-5}$	$8\ 92 \times 10^{-6}$	$3\ 16 \times 10^{-1}$	$1\ 57 \times 10^{-6}$
4	$1\ 79 \times 10^{-6}$	$2\ 24 \times 10^{-7}$	$2\ 24 \times 10^{-1}$	$6\ 08 \times 10^{-7}$
5	$6\ 32 \times 10^{-8}$	$8\ 92 \times 10^{-9}$	$1\ 12 \times 10^{-1}$	$4\ 49 \times 10^{-8}$
6	$1\ 13 \times 10^{-8}$	—	—	$4\ 13 \times 10^{-8}$

The observed values of N_J/N_0g_J for three stars[6] have also been included for comparison Table 2 shows that the agreement between the calculated and observed values of N_J is not unsatisfactory for the stars ζ Oph and ε Per For ρ Pup, no agreement is possible and excitation of the rotational levels of H_2 has to be by process other than excitation by collisions with grains

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Cosmological absorption of gravitational waves

SEVERAL people have investigated the absorption of electromagnetic and neutrino waves in cosmological models and discussed the self-consistency or otherwise, in the Wheeler–Feynman absorber theory of radiation, of retarded and advanced fields, a solution of the field equations is self-consistent if the absorption tends to completeness as the field propagates Cosmological absorption of gravitational waves has been treated briefly by Hawking[1] Here, the completeness or otherwise of the absorption of retarded and advanced gravitational waves will be investigated using a technique introduced, in the case of electromagnetic absorption, by Davies[2], the technique applies to the Robertson–Walker cosmologies of arbitrary spatial curvature

In a cosmological model with scale factor R, consider a wave that has angular frequency ω relative to a comoving

observer, $\omega = \omega_i/R$, a subscript i denoting the value of a quantity near the source of the wave Let t' be a time coordinate for which the metric is proportional to that of special relativity Take a time factor $e^{i\omega t'}$ and let $\varepsilon = -1$ and $+1$ for retarded and advanced fields respectively Provided the refractive index does not vary too rapidly with the radial coordinate r, the absorption factor is

$$\exp[(\varepsilon\omega_i/c) \int k\,\mathrm{d}r] \tag{1}$$

where c is the speed of light in free space, k is the imaginary part of the refractive index and the integration is over the path of the wave For fields propagating on light cones $\mathrm{d}r = -\varepsilon c\mathrm{d}t'$ and for cosmological models that are spatially flat t' is related to the cosmic time t by $\mathrm{d}t/\mathrm{d}t' = R$, so that expression (1) becomes

$$\exp(-\int \omega k\,\mathrm{d}t) \tag{2}$$

For gravitational waves, this factor is[1]

$$\exp[-(G/2c^2) \int \eta\,\mathrm{d}t] \tag{3}$$

where G is the Newtonian gravitational constant and η is the coefficient of viscosity of the medium

For atomic hydrogen $\eta \sim 10^{-4}T^{1/2}$ g cm^{-1}s^{-1} where T is the absolute temperature For fully ionised hydrogen in the absence of a magnetic field[3] $\eta \sim 10^{-16}T^{5/2}$g cm^{-1}s^{-1}

Consider absorption in a static homogeneous universe The factor (1), with $k = G\eta/2c^2\omega$, shows that appreciable absorption will occur for propagation over a range d of r where $d \approx c^3/G\eta$ If H denotes the Hubble constant of the actual Universe, then a characteristic distance in cosmology is c/H, which is about 10^{28} cm If $d \gtrsim 10^{28}$ cm, then the static model is inadequate to treat cosmological absorption In the actual Universe, observations have shown that the intergalactic medium could consist of fully ionised hydrogen with[4] 6×10^8 K$> T > 10^4$ K, corresponding in the static model to $d \sim 10^{33}-10^{45}$ cm

Suppose that all matter in the universe can be represented by a smoothed number density N Consider cosmological models of Class I in which there is continuous creation of matter at such a rate as to keep N constant, and models of Class II, in which matter is conserved so that $N \propto R^{-3}$ As an advanced wave propagates into the past its wavelength decreases and, for Class I models, eventually becomes comparable with the mean free path of the particles of the cosmic medium, then the above formulae for the viscosity are inapplicable Those formulae also fail eventually for retarded waves in Class II models, since the mean free path increases more rapidly than the wavelength When the mean free path greatly exceeds the wavelength, so that the particle collision frequency ν is much less than the wave frequency, for a non-relativistic medium[5] $k \sim N\nu T/\omega^3$

Let σ_e denote an effective cross section equal to $\omega k/Nc$ Absorption will be complete for retarded and advanced fields in the Robertson–Walker cosmologies if[2]

$$\left| \int^\infty N\sigma_e \, \mathrm{d}t \right| = \infty \tag{4}$$

and

$$\left| \int^0 N\sigma_e \, \mathrm{d}t \right| = \infty \tag{5}$$

respectively For gravitational waves expressions (3), (4) and (5) show that $N\sigma_e = G\eta/2c^2$ Take $R \propto t^n$ with $n > 0$ and consider retarded gravitational waves In Class I universes, if the temperature of the cosmic medium is constant, then expressions (3) shows that absorption is complete for all n In Class

II universes, take $k \sim NvT/\omega^3$ Also, take $v \sim NT^\alpha$ where α is a constant, thus $N\sigma_e \sim N^2 T^{\alpha+1}/\omega^2$ Suppose that $T \sim R^{-\beta}$ in the distant future From equation (4), absorption is complete if $[4+(\alpha+1)\beta]n \leqslant 1$ Davies[2,6] investigated the thermal future of the universe by considering the interaction of the intergalactic medium with the cosmic electromagnetic blackbody background radiation He showed that for models that expand more slowly than $t^{1/2}$, the intergalactic plasma eventually completely recombines, for models that expand more rapidly than $t^{1/2}$, the plasma does not completely recombine and remains coupled to the electromagnetic background radiation If the intergalactic medium eventually recombines, then $\alpha = 1/2$ and $\beta = 2$, if the medium does not recombine, then $\alpha = -3/2$ and $1 \leqslant \beta \leqslant 2$ Thus, absorption of retarded gravitational waves in Class II universes is complete if $n \leqslant 1/7$

Now consider advanced gravitational waves Assume that σ_e approaches a constant as $\omega \to \infty$ In Class I universes, expression (5) shows that absorption is finite for all values of n In Class II universes with a "hot big-bang", as they travel into the past, advanced waves that are not absorbed first eventually enter an epoch in which the cosmic matter is in thermal equilibrium with electromagnetic radiation at relativistic temperatures As this epoch is entered there is a rapid increase in N because of thermal electron–positron pair production[7], within this epoch the mass density of the cosmic medium varies as R^{-4}, but N still varies as R^{-3} Thus full absorption of advanced waves occurs for $n \geq 1/3$

Consider now condensed objects, and suppose that their interaction with gravitational waves can be represented by a constant σ_e Suppose that in Class I universes there is a constant number density of such objects for retarded waves absorption is complete, for advanced waves, absorption is finite Suppose that in Class II universes the number density of condensed objects varies as R^{-3} in the distant future absorption of retarded waves is complete for $n \leqslant 1/3$

Assume, for the sake of argument, that the absorber theory of radiation is valid for gravitational waves, that only retarded gravitational waves can be observed in the actual universe and that σ_e saturates at high frequencies In determining what cosmological models are to be eliminated, with these assumptions, as possible models of the Universe, the relevant values of n are those applicable for $t \to 0$ and for $t \to \infty$, such values will be denoted by n_0 and n_∞ respectively No Class I universe with the characteristics taken above is eliminated Class II universes with $n_0 > 1/3$ are eliminated Class II universes with $n_\infty > 1/3$ are eliminated In the standard "hot big-bang" model of general relativity, $n = 1/2$ in the radiation-dominated epoch so that this model has consistent advanced fields and is eliminated In the spatially flat case of the Brans–Dicke cosmology, $n = (2\omega_{BD}+2)/(3\omega_{BD}+4)$ where ω_{BD} is a parameter, with[8] $\omega_{BD} \geq 0$ it follows that $1/2 \leqslant n \leqslant 2/3$, where the upper limit corresponds to the Einstein–de Sitter universe of general relativity these models are eliminated since retarded fields are inconsistent The Friedmann models with zero cosmological constant and negative spatial curvature have $n_\infty = 1$ and are hence eliminated

Of course, these eliminations are purely hypothetical, being based on the three assumptions mentioned above If cosmological observations were to establish one of these hypothetically eliminated models as a reasonable model of the Universe, then that result would constitute evidence against at least one of the assumptions

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Solar test of Dirac's large numbers hypothesi

DIRAC[1,2] has proposed that the large dimensionless numbei that are constructable from the fundamental constants c physics and astronomy are related to each other, and ai simple functions of our present epoch in the Universe Thus h suggests that, as measured by atomic standards, the gravita tional constant, G, varies with the time as t^{-1} and the number c nucleons in the Universe increases as t^2 In more recent work[3,] he has pursued the consequences of assuming different mode of creation of new matter We have found that Dirac's theory c multiplicative creation, but not his theory of additive creatioi is not in contradiction with known facts about the Sun

By "additive creation" he implies that matter is forme uniformly throughout space, and therefore mostly in inte, galactic regions, the mass of a small object like a star woul remain essentially unaltered by the new creation of matte "Multiplicative creation" means that existing matter multiplie itself in proportion to the amount of matter already present therefore the mass of a star, M, would grow as t^2

In view of the important cosmological consequences c Dirac's theory, it is desirable to test it with methods present available One useful test object is the Sun The proposed larg change in G over the Sun's past lifetime and, with multiplicativ creation, the concomitant large change in the Sun's mass, woul be expected to produce a different evolutionary history for th Sun as compared with the 'standard' history computed on tl basis of constant G and M But with any theory the observab properties of the Sun today (mass, luminosity, and radiu must be achievable with 'reasonable' choices of initial chemic composition Unfortunately, the chemical composition of th present surface layers, which presumably reflect the origin composition, is known only with great uncertainty, and th allows a considerable latitude of choices A further constrair (and a possible benefit that could accrue from a 'non-standar theory) is the small solar neutrino flux that is observed and not explained by 'standard' theories

The one 'free' parameter in Dirac's theory is the age of tl Universe, t_0, although observations of the Hubble constant p some limits on it If t is measured from the time of formatic of the Sun, then

$$G(t) = G_0 \left[\frac{t_0 - t_\odot + t}{t_0} \right]^{-1}$$

and

$$M(t) = M_0 \left[\frac{t_0 - t_\odot + t}{t_0} \right]^n$$

with $n=0$ for additive creation and $n=2$ for multiplicati creation At the present epoch, $G_0 = 6\ 67 \times 10^{-8}$ cm^3 g^{-1} s $M_0 = 1\ 99 \times 10^{33}$ g, and $t_\odot = 4\ 5 \times 10^9$ yr

The hypothesis of additive creation has already been tested Pochoda and Schwarzschild[5] They found that this hypothe can be satisfied if $t_0 \geqslant 15 \times 10^9$ yr Today, such an age for th Universe is certainly admissible, although it seemed rather long a decade ago But the required initial hydrogen conte $X = 0\ 81$, seems somewhat high Moreover, the predic brightness of the Sun in the past, in spite of the greater dista

of the Earth from the Sun then (in the additive theory), may conflict with palaeontological evidence[6] One further consequence of the additive theory is particularly relevant here the hydrogen content near the centre of the present Sun must be completely exhausted Although Pochoda and Schwarzschild did not publish any temperatures, the central temperature of their final model is probably about 19×10^6 K (see, for example, ref 7) In these conditions the expected neutrino flux must be enormous—several orders of magnitude greater than the upper limit of the neutrino flux measured by Davis[8] But it should be borne in mind that standard solar models themselves predict a neutrino flux that is about an order of magnitude larger than Davis's upper limit of 1 SNU

In order to test Dirac's hypothesis of multiplicative creation, we have evolved several sequences of stellar models up to the present age and mass of the Sun It has been assumed that, since the solar gases are highly ionised, new ions (and a corresponding number of electrons) are created in the vast empty spaces between the existing particles They are assumed to be of the same species as their 'parent' ions Moreover, they must share the same kinetic energy and linear momentum as their neighbours, on the average, and therefore units of kinetic energy and momentum (including units of rotational angular momentum of the whole Sun) must also be created Dirac has already postulated these conditions for the Earth, in order not to contradict geological evidence For our purposes, it is sufficient merely to scale up the chemical structure of the evolving solar models as the mass of the Sun is increased For simplicity, axial rotation of the Sun is neglected

The other physical input parameters are the same as those used in previous work[9] To summarise them here for convenience, we have adopted the new Carson (unpublished) opacities, which are very close to those of Cox and Stewart[10] for main-sequence stars of low and intermediate masses, the nuclear cross sections of Bahcall, Bahcall and Ulrich[11], but with the neglect of the *pep* reaction, a ratio of mixing length to pressure scale height in the outer convective envelope equal to $\alpha = 2$, and a metals abundance of $Z = 0\,015$ Results for the initial and final solar models are given in table 1 We have found that the final radius does not agree exactly with the observed radius, for the choice of $\alpha = 2$ But, as many people have shown a suitable change of α can achieve agreement between the radii without significantly altering the luminosity, which is affected primarily by the choice of initial chemical composition Therefore, we have not considered it worthwhile to rerun the sequences with different guesses for α until the final radii are correct

The standard solar model obtained here is very similar to the analogous one already calculated by Carson, Ezer, and Stothers[9] The two models are not identical because we have here adopted equilibrium nuclear reaction rates and a slightly different treatment of the thermodynamical quantities, apart from the fact that the computer program itself is different

Turning now to the final solar models based on Dirac's multiplicative theory, we find the rather surprising result that they are nearly the same as the final model based on standard

theory[1] This occurs in spite of the widely disparate initial masses, occasioned by the use of a full range of choices for t_0 The reason for this similarity is that the effect of a larger G in the past is to increase the luminosity[6], whereas a lower stellar mass decreases it As a result of these compensating factors, the ratio L/M remains approximately constant Since the nuclear reaction rate and the amount of hydrogen depletion depend on the L/M ratio[12], the final central temperature and the final hydrogen profile throughout the Sun are about the same as they were in the standard case

Since the radius, r, of the Earth's orbit around the Sun increases, in Dirac's multiplicative theory, as t, the temperature of the Earth's surface may be expected to change like $(L/r^2)^{1/4}$ ~ constant This naive prediction is not in contradiction with palaeontological evidence But why are well-preserved Precambrian and early Cambrian fossils in essentially perfect shape if their masses have increased by a significant percentage?

One could pursue the test of Dirac's theory by constructing theoretical isochrones for old clusters containing stars of low mass and comparing them with observed cluster H–R diagrams Although we have not done this, it is interesting that the more massive members of this old population must have evolved long ago—mostly into white dwarfs, initially But since the upper mass limit for a stable white dwarf (or neutron star) is proportional to $G^{-3/2} \sim t^{3/2}$, and since the mass of the star itself increases as t^2, eventually these stars will all undergo gravitational collapse The galactic halo must be popping with continually forming black holes The ultimate cosmological consequence is a universe full of gravitationally collapsed objects

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Table 1 Final models for the present Sun based on Dirac's theory of multiplicative creation

Case	Standard	1	2	3
t_0 $(10^9$ yr)	—	20	10	7
Initial G/G_0	1	1 29	1 82	2 80
Initial M/M_0	1	0 60	0 30	0 13
X	0 758	0 755	0 751	0 749
Z	0 015	0 015	0 015	0 015
Initial log $L/L_\odot$	−0 13	−0 43	−0 83	−1 35
Initial log T_e	3 73	3 71	3 67	3 62
Final X_c	0 40	0 43	0 45	0 47
Final T_c $(10^6$ K)	15 2	15 1	15 1	15 0
Final ρ_c (g cm^{-3})	151	144	140	133
Neutrino flux (s n u)	~7	~6	~6	~5

Age and rates of denudation of Trap Series basalts at Blue Nile Gorge, Ethiopia

BETWEEN Lake Tana and the Sudan border the Blue Nile has cut a deep gorge across the Ethiopian Highlands The first 150 km of its course lies on lavas of the Tertiary Trap Series, after which the river flows through horizontal Mesozoic and Palaeozoic sediments until the Precambrian is reached 330 km from Lake Tana[1] About 280 km below the lake the Addis Ababa–Debre Markos road crosses the gorge at 10° 05'N, 38° 10'E, where 250 m of Trap Series basalts overlie at least 1,150 m of sediments the base of which is not exposed The gorge is 1,400 m deep and 20 km wide and is incised into a plateau 2,600 m high

On the south side of the gorge the Trap Series section comprises at least five thick horizontal flows of aphanitic basalt, belonging to the lower part of the Trap Series as a whole The top three flows are well exposed and each is several tens of metres thick and extends laterally for over

Table 1 Potassium–argon ages on whole rock samples of basalt from the left bank of the Blue Nile Gorge near the crossing of the Addis Ababa–Debre Markos road, Ethiopia

Field location	Laboratory no	Weight %	Rad ^{40}Ar $(10^{-7}$ cm^3 NTP g$^{-1})$	$\dfrac{100 \text{ Rad } ^{40}\text{Ar}}{\text{Total } ^{40}\text{Ar}}$	Calculated age (Myr)±2 s d	Approximate percentage glass or mesostasis
Top flow	72–555	0 739, 0 743	6 99	62 7	23 5±0 3	30
Second flow	73–667	0 919, 0 925	8 97	82 1	24 2±0 4	15
Third flow	73–668	0 776, 0 772	7 15	78 1	23 0±0 3	20
Fourth flow	73–669	0 888, 0 891	9 73 / 9 80	84 6 / 84 0	27 2±0 4 / 27 4±0 4	10

$\lambda_e = 0\ 585 \times 10^{-10}$ yr^{-1}, $\lambda_\beta = 4\ 72 \times 10^{-10}$ yr^{-1}, ^{40}K/K $= 1\ 19 \times 10^{-2}$ atom %

5 km These features suggest that the flows were the product of fissure eruptions[2]

Grasty et al[2] tried to date the flows, but their results were inconclusive We have dated fresh samples from the top four flows (Table 1), using techniques described previously[3] It was not possible to obtain samples free from interstitial glass or mesostasis, and the possibility of argon losses must be taken into account when interpreting the results, notably the low age obtained for the third flow Nevertheless, the close agreement in ages obtained suggests strongly that they are near the truth, and that the actual age of the basalts is certainly less than 30 Myr This age is similar to the ages obtained[4] from the lower part of the Trap Series elsewhere in Ethiopia

The dating of these horizontal flows gives a lower limit to the age of incision by the Blue Nile into the uplifted Ethiopian highlands Merla[5] considered that uplift occurred in two stages one in the Miocene, and the other in the Plio–Pleistocene with the development of deep canyons, including the Blue Nile and Tekezze (Atbara) gorges The overall rate of erosion since the deposition of these basalts may be calculated by two methods One is to find the volume of sediments derived from the upland Blue Nile and Tekezze basins and deposited in the Gezira and Atbara fans, along the main Nile and in the Nile Delta The other is to calculate the volume of rock eroded from the uplands themselves

At present the White Nile provides less than 2% of the annual sediment load of the main Nile[6], and the high proportion of Ethiopian minerals[7] and pollen[8] in Nile deltaic sediments indicates that a similar situation obtained during at least part of the Cainozoic Various estimates of the volume of sediments comprising the Nile Delta have been made[9,10], but taking into account seismic work[11], a figure of between 100,000 and 200,000 km^3 seems reasonable The volume of alluvium along the main Nile flood-plains and terraces between Khartoum and the delta is not known, but an estimate of between 100 and 600 km^3 can be made The Gezira Plain between the Blue Nile and the White Nile south of Khartoum is a low-angle alluvial fan built up mainly by the Blue Nile[12] Isopach values[13] indicate a volume of about 1,800 km^3, which excludes probable White Nile alluvium A rough estimate of 800 km^3 may be made for the volume of the Atbara Fan, given its smaller catchment area relative to the Blue Nile

The total volume of material deposited by the Blue Nile and Atbara (Tekezze) in Egypt and Sudan is, therefore, estimated at between 98,000 and 225,000 km^3, the bulk of which forms the Nile Delta For simplicity we here assume a value of $150,000 \pm 50,000$ km^3, derived mainly from Ethiopia The areas of the two basins in upland Ethiopia above 1,000 m are 190,000 and 85,000 km^2, respectively Allowing for the difference in bulk density between Nile deltaic sediments (about 1 5 g cm^{-3}) and the eroded Ethiopian bedrock (about 2 8 g cm^{-3}), and assuming a 30% loss in solution from the Ethiopian uplands, the mean erosion rate over the past 23 5 Myr would be 12 ± 6 m^3 km^{-2} yr^{-1}

This result may be compared with that obtained by calculating the volume of rock eroded from the two basins, using a contour map of the highlands The dissected plateau has a mean local relief of about 1,500 m and a mean surface elevation of about 2,500 m The estimated volumes of rock removed from the Blue Nile and Tekezze basins are 71,000 km^3 and 31,000 km^3, respectively That gives a total of 102,000 km^3, to an accuracy of about 50% As the area of the basins is about 275,000 km^2, the mean rate of denudation must have been $15 \pm 7\ 5$ m^3 km^{-2} yr^{-1}

Such denudation rates of around 0 015 mm yr^{-1} are very low for a tectonically active region of high relief[14–18], they are more in keeping with modern rates from undisturbed, forested, tropical lowlands[19–21] Modern erosion rates in the region are far higher, with estimates of the Nile sediment load at Cairo ranging from 57 to 120 Mt yr^{-1} (refs 6, 22 and 23) These estimates show a doubling of sediment load during the past 70 yr[23,24], reflecting accelerated erosion of the headwaters If an annual sediment load of 50–100 Mt is derived from Ethiopia, the present rate of erosion in the headwaters of the Blue Nile and Tekezze must be about 120–240 m^3 km^{-2} yr^{-1}, or an order of magnitude greater than the overall geological rate

The discrepancy between the two rates suggests episodic uplift interspersed between prolonged stable intervals Faure[25] calculated the rate of uplift of the plateau to be 0 5–1 0 mm yr^{-1} during the Upper Pleistocene, compared with a mean rate of 0 1 mm yr^{-1} since the Middle Tertiary We conclude that a change in the rate of uplift of that magnitude, coupled with recent deforestation of the head-waters, can explain adequately the difference between the modern and geological erosion rates It may be further noted that the close agreement between the volume of material eroded from Ethiopia, and the volume in the Nile Delta, is further evidence suggesting that the bulk of the Nile deltaic sediments are of Ethiopian provenance

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Non-etching optical detection of fission tracks using Teflon
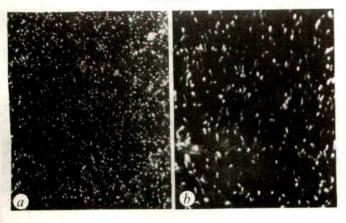
THE detection of tracks in material irradiated by charged particles, has been accomplished using electron microscopy[1] and etching followed by optical microscopy[2-6]. We have attempted to detect unetched tracks using optical microscopy. Presumably, unetched tracks retain more information about the radiation than is retained after etching.

The kinds of damage which can be caused by the passage of a charged particle through any material are bond ruptured, which produces free radicals[7], physical dislocations, as in the Wigner effect in reactors in which carbon atoms in graphite are displaced by fast neutrons, and certain other less obvious but more visible effects such as the production of metastable states which emit light, as in thermal luminescence and scintillation counters.

It seemed possible that, in order to obtain the best record of the track itself, a material and detection process could be used which would minimise the general background and make possible the direct observation of the track itself using an optical microscope. So we chose, following the work of others[8-12], to use cross linkage copolymerisation of radiation-damaged polytetrafluoroethylene, Teflon, with acrylic acid monomer followed by dying with the basic dye Rhodamine B which phosphoresces in orange under exposure to blue light. Teflon is one of the most chemically resistive materials known and should give essentially no background.

Teflon plastics films (51, 127, and 762 μm thick) were irradiated in air with fission products from a 50 μCi ^{252}Cf source for 1–2 min at room temperature. The samples were then inserted into glass tubes containing acrylic acid solution and degassed by freezing and pumping the system

Fig. 1 Tracks of fission fragments. *a*, × 180 diameters; *b*, × 360 diameters. Made by grafting acrylic acid on to Teflon. The apparent length of each track is related to the angle of its inclination.

several times. After degassing, a grafting reaction with the free radicals in the tracks was allowed to continue in the absence of air at controlled temperatures (23–40 °C), for varying times (5–30 h). Good grafting was obtained at 23 °C after 25 h or at 40 °C after 5 h. We were also successful in getting good grafting results when the reactions were carried out in a dry nitrogen atmosphere. In these experiments dry nitrogen gas was bubbled through the acrylic acid solution for 15 m and then the tube was closed. Grafting occurred in dry nitrogen under conditions similar to those used in the evacuation experiments.

The solution was 50 % vacuum distilled acrylic acid ($CH_2.CH.COOH$). After the grafting, the Teflon samples were washed thoroughly in deionised water at 50 °C for 2 h. Following this they were dyed with a boiling 3 % solution of Rhodamine B for several hours and then washed for 15 min in a 2 % acid soap solution to remove any unfixed dye. This procedure was apparently successful: the basic dye fixed itself to the acidic polymer that was attached to the damaged area of the Teflon.

Finally, the samples were examined with a Reichert-Austria Zetopan microscope fitted with an ultraviolet lamp. Rhodamine B phosphoresces with an orange colour when illuminated with light at a wavelength of 400 nm. A cutoff filter eliminated the excess blue light which produced a dark field, and the fission tracks then appeared as bright lines (Fig. 1a and b).

It seems possible that more information can be obtained from tracks developed and measured in this way and that Teflon treated like this may well be useful in the future detection of tracks formed by the passage of charged particles.

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Rapid hydrogenation of sterols in a contemporary lacustrine sediment

THE steroid carbon skeleton has been widely used as an indicator of biological origin of the organic material in ancient sediments[1-3] and petroleum[4]. Sterols, presumed precursors of the geolipid steranes, and stanols, their saturated analogues, have been identified in Recent[5-7] and ancient[8,9] sediments. In view of their generally low abundance in living

organisms, the presence of stanols in Recent sediments suggests the operation of a sterol hydrogenation process early in the geological time scale. We have sought to confirm this hypothesis by examination of the contemporary sediments of Rostherne Mere, Cheshire, England. The sterol composition of the sediment, and its variation with depth, provide circumstantial evidence for the hydrogenation of Δ^5-sterols[10]. Here we describe the application of radiochemical techniques[11,12] to the study of the early diagenesis of cholesterol (cholest-5-en-3β-ol) in Rostherne sediment and report the first direct evidence of rapid sterol hydrogenation in a geological environment. The sediment chosen for study is that of a highly productive lake. Significant allochthonous (land-derived) organic input to Rosterne Mere has been exceeded in recent years by an increasing autochthonous production of blue-green algae[13,14]. The sediments in the deepest part of the lake (from where samples were taken) remain severely depleted in oxygen throughout the year[15]. As a National Nature Reserve, Rostherne Mere is protected from gross pollution, though there is some chemical evidence to suggest a recent input of petroleum-derived material[14].

4-[14]C-cholesterol (Radiochemical Centre, Amersham) was purified by thin-layer chromatography on silica gel and on silver nitrate impregnated (30% by weight) alumina (Ag$^+$TLC)[16], to remove traces of [14]C-stanols. Incubations with 4-[14]C-cholesterol were conducted both in sediment cores from Rostherne Mere and in the model environment of anaerobic sewage sludge. Sediment cores (50 cm × 6 cm), encased in Perspex tubes, were taken intact from the deepest part of Rostherne Mere. For laboratory incubation, the core was stored in a polystyrene container, over a small quantity of dry ice, during transport to the laboratory. A second sediment incubation involved injection of 4-[14]C-cholesterol and suspension of the core in the lake near the sediment–water interface for the duration of the incubation. The "zero time" incubation, in which acidification and extraction (heptane–isopropanol) immediately followed injection of radiolabel, enabled an assessment of purification and work-up procedures. Further experimental details are given in Tables 1 and 2; a full discussion will be provided elsewhere (S. J. G. and G. E., in preparation).

Table 1 summarises the sterol constituents identified in Rostherne sediment. Examination of the variation in sterol composition indicates an increase in the stanol to Δ^5-sterol ratio with sediment depth (ref. 10 and S. J. G. and G. E., in preparation). For the 0–10 cm sediment level, examined in conjunction with a labelling experiment (A, Table 2), the carbon number distributions of the Δ^5-sterol, 5α-stanol and

Table 2 Incubation of 4-[14]C-cholesterol in Rostherne sediment and anaerobic sewage sludge

Radiolabel	Experiment*			
	A	B	C	D
	(sediment)	(sediment)	(sewage)	(sewage)
Total activity injected (μCi)†	14.67	3.10	3.61	3.23
Incubation products:‡				
total lipids (%)	69	29	77	75
[14]C-cholesterol (%)	45	nd §	28	68
[14]C-5α-cholestanol (%)	0.47	0.09	0.66	—¶
[14]C-5β-cholestanol (%)	0.11	0.03	1.2	0.06

* A, Incubation (90 d, 10 °C) in intact core in the laboratory (dark), injection at 4 cm sediment depth; B, incubation (65 d, 5.4 °C) at lake bottom, injection at 4 cm sediment depth; C, incubation (28 d, 37 °C) in the laboratory (dark); D, "zero-time" incubation (see text).
† Injections of 4-[14]C-cholesterol made in Tween 80 suspension.
‡ Expressed as percentage of radiolabel injected; no allowance made for experimental losses. Total extracts separated by shaking with aqueous KOH (7%) and neutrals further separated by silica TLC. "Δ^5-sterols", "5α-stanols" and "5β-stanols" obtained from crude total sterol fraction by Ag$^+$TLC. Radiolabelled stanol products of incubation characterised by radio-TLC, radio-GLC and GC–MS (S.J.G. and G.E., in preparation). Other radiolabelled products uncharacterised or not fully characterised; see text.
§ Not determined.
¶ Radioactivity not detected above background.

5β-stanol constituents are similar, confirming the earlier findings[10]. The presence of large quantities of stanols, relative to unsaturated sterols, together with the parallel distributions of saturated and unsaturated analogues provides strong evidence for the operation of an hydrogenation process in the young Rostherne sediment.

The results of radiolabelling experiments are summarised in Table 2. The incomplete recovery of radiolabel in the zero time incubation experiment D emphasises the difficulty of quantitative extraction of sterols. The particularly low recovery in experiment B, where incubation was conducted in the lake, may be attributable to losses at the flocculent sediment–water interface. In sediment incubations, unchanged [14]C-cholesterol was the major labelled product but sewage incubation (experiment C) yielded a major fraction (31% of injected radioactivity) corresponding in polarity to unsaturated steroid ketones (see below). Each stanol fraction, obtained by Ag$^+$TLC, was subjected to a second Ag$^+$TLC separation to ensure absence of [14]C-cholesterol. The "5α-stanol" TLC fraction from zero-time incubation (D, Table 2) contained no detectable activity. The origin of the small quantity of activity in the "5β-stanol" fraction is uncertain but may arise from TLC overlap with products of sterol dehydration during the work-up procedure. The levels of activity in the stanol fractions from sediment and sewage incubations (A and C, Table 2), however, indicate clearly that both 5α- and 5β-stanols are genuine products of incubation. In experiments A and C, analyses by radio-gas chromatography and combined gas chromatography–mass spectrometry (GC–MS) established [14]C-5α-cholestanol and [14]C-5β-cholestanol as the sole radiolabelled components of their respective fractions. The 5α-isomer predominated, both as a radiolabelled product of incubation and as a natural constituent, in the sediment incubation whereas 5β-cholestanol was the more abundant in sewage sludge.

In both sediment and sewage incubations, other labelled products of incubation included components whose TLC mobilities were consistent with saturated and unsaturated steroid ketone structures. Radio-gas liquid chromatography (GLC) analyses with coinjection with authentic standards suggested the presence of [14]C-cholest-4-en-3-one, [14]C-5α- and [14]C-5β-cholestanone. This evidence suggests that the hydrogenation of the Δ^5 double bond in cholesterol, effective in sediment and sewage sludge, may be a biochemical process for which the mechanism is similar to that observed to be

Table 1 Sterol constituents of Rostherne sediment*

Structural type†	Abundance (p.p.m.) of constituent‡		
	C27	C28 (24-methyl)	C29 (24-ethyl)
Δ^5-sterol	*20*	*11*	*24*
$\Delta^{5,22}$-sterol	*1*	*7*	*5*
Δ^{22}-sterol (5α)	?	*10*	*8*
5α-stanol§	*47*	*22*	*49*
5β-stanol	*9*	*3*¶	*9*

* Constituents of a 0–10 cm sediment core (experiment A, Table 2). Individual sterols identified on the basis of gas chromatography (GC) retention times (trimethylsilyl ethers) and mass spectra (obtained by gas chromatography–mass spectrometry (GCMS) of the trimethylsilyl ethers facilitated by computer accumulation and interpretation of data[14,17,18]). Authentic standards available for those components for which amounts are given in italics.
† Δ^5-sterols (with $\Delta^{5,22}$-sterols) separated from 5α-stanols (with Δ^{22}-sterols) and 5β-stanols by Ag$^+$TLC. Fraction most strongly retained on Ag$^+$TLC not examined.
‡ Constituents designated by carbon number. "?" indicates lack of positive identification. Abundances expressed as p.p.m. dry weight of extracted sediment.
§ C28 stanol observed at lower sediment depth (ref. 10, and S.J.G. and G.E., in preparation).
¶ Tentative assignment.

operative in certain bacterial[19,20] and other biological systems[21,22].

We believe that the results of the radiolabelling experiments with [14]C-cholesterol in Rostherne sediment provide a clear indication of an hydrogenation process taking place in the sediment under environmental conditions, for the following reasons. First, the radiolabelling results are consistent with the indirect evidence for a sterol hydrogenation process based on sediment lipid analyses. The relative amounts of Δ^5-sterols and 5α-stanols (in approximate ratio 1:2), as natural sediment constituents, suggest a conversion of approximately 60% during the period of deposition of the 0–10 cm sediment layer (about 20 yr), indicating an average rate of hydrogenation approximately equal to that observed in the labelling experiment (A) (that is, 0.5% in 3 months).

Second, the specific activities (determined by radiocounting and GLC quantitation, with an internal standard) of radiolabelled 5α-cholestanol and 5β-cholestanol, following incubation of [14]C-cholesterol in Rostherne sediment (experiment A) were the same (0.11 μCi mg^{-1}).

Third, incubation of [14]C-cholesterol in a sediment core, suspended in the lake for the duration of the incubation (experiment B), also indicates conversion to radiolabelled stanols.

These experiments have demonstrated that the geochemically significant hydrogenation of naturally-occurring unsaturated sterols, to give both 5α- and 5β-stanols, may be effected extremely rapidly in contemporary aquatic sediments. The presumably microbiological process may be analogous to that observed in other biological systems with the consequent 5α- : 5β-stanol ratio dependent on the particular microbial population (compare the results of [14]C-cholesterol incubation in anaerobic sewage sludge).

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The hipparions of the Baringo Basin sequence

THE first appearance of Hipparion in land mammal populations of the northern hemisphere is one of the best documented biochronological events of the Neogene: the genus appeared first in the Mediterranean world about 12.5 Myr ago[1]. In France[2] its first appearance was during the Middle–Upper 'Helvetian' (ante-'Tortonian'). New radiometric information from the Far East supports an age of 10.5 Myr for the base stratotype Tortonian[1]. This means that the Hipparion datum of 12.5 Myr is some 2 Myr earlier than the advent of the Vallesian.

In North Africa the earliest Hipparion is the same as the primordial Old World Hipparion primigenium (Von Meyer) of the 'Pontian' (Vallesian and Pikermian) of Europe. It was described as Hipparion africanum Arambourg[3] from the Vallesian of Wad el Hammam in Algeria[4]. H. primigenium also occurs in the Tortonian Beglia Formation of Tunisia[5].

In Asia, Hipparion appeared first in the Nagri Formation of the Siwaliks[6] about 10–12 Myr ago. Hussain described the Nagri Formation Hipparion as Hipparion nagriensis[7]. Although the skull is unknown, H. nagriensis seems to be a clear synonym of Hipparion primigenium: the cheek teeth are of the same size and have the very characteristic pattern of this species (see ref. 7 plates 1 and 2, and ref. 8 Figs 21 and 22). Hussain noted the resemblance of H. nagriensis to Hipparion catalaunicum Pirlot and H. koenigswaldi Sondaar[7], both of which are synonyms of H. primigenium[4].

In the summer of 1973 I received the Hipparion remains from the Ngorora Formation in Kenya, which is between 12 and 9 Myr old[9] (see also ref. 10). Before this discovery, it was thought that the earliest Hipparion south of the Sahara was in the Mpesida Beds of Kenya, dated at 7 Myr, terminal Pikermian[9,11]. The Hipparion from the Mpesida Beds is the same as that from Lothagam–1 in the Turkana District on the west side of Lake Rudolf in Kenya, age 6 Myr. It has been described as Hipparion turkanense[12,13].

The Ngorora Hipparion is very different from H. turkanense but is indistinguishable from Hipparion primigenium. Hipparion primigenium also occurs in the Chemeron Formation (locality J.M.493), at Kanapoi, and in the Aterir Beds. On the other hand, teeth indistinguishable from H. turkanense are found in the Kaperyon Beds. Thus, it seems that there are two large species of Hipparion in the Baringo Basin sequence: H. primigenium emerging in the Ngorora Formation, and H. turkanense in the Mpesida Beds, representing a later invasion from Asia. The two species survived till the 4 Myr level, for teeth indistinguishable from those of H. turkanense are found at Kanapoi and in the Mursi Formation of southern Ethiopia. With the discovery of Hipparion primigenium in the Ngorora Formation it has been settled that there is not so much of a time lag between the first appearance of Hipparion south of the Sahara and that north of the Sahara as had been supposed previously. One species only, Hipparion primigenium (Von Meyer), first appears to be distributed over much of the Old World, not only in Eurasia but also in Africa both north and south of the Sahara in the Vallesian.

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Distribution of hydrogen bond angles in molecular crystals

THE structures of hydrogen-bonded molecular crystals have been studied extensively, and the resulting histograms of the distributions of the O–H $\cdots$ O bond angles θ show maxima at approximately 15° from the linear configuration[1-3]. At first sight this seems odd, because theoretically a linear hydrogen bond is stable[4,5]. Kroon and Kanters[3] have indicated, however, that because the number of possible hydrogen bond configurations at any value of θ is proportional to $\sin\theta$, these histograms should not be interpreted to indicate that the configuration of $\theta = 15°$ is, energetically, the most stable. They applied the $\sin\theta$ correction to the statistics of a series of 196 values of hydrogen bond angles in molecular crystals, and showed that the experimental distributions are not inconsistent with an assumed preference for linear hydrogen bonds.

One of us (M.H.)[5] has calculated the potential energy function for the bending of the hydrogen bond O–H–O $\cdots$ O–H$_2$ using the method of CNDO/2, one of the semi-empirical LCAO MO SCF methods which includes all the valence electrons. We intend to show here that the histograms of Kroon and Kanters[3] can be explained by Hasegawa's[5] potential energy function.

The potential function[5] can be represented approximately by the following quadratic form:

$$\Delta E = s\theta^2/2 \tag{1}$$

in which the linear hydrogen bond is most stable and ΔE represents the strain energy for the bending through θ degrees of the hydrogen bond. The value of the force constant, s, is estimated at $0.4\,\text{eV}/\text{rad}^2$. In the case of the hydrogen-bonded water dimer, the dependence on the angle ω of the strain energy ΔE is small, so that factor is neglected here.

If it is assumed that the distribution among configurations in various molecular crystals may be approximated by a Boltzmann distribution, the distribution function $f(\theta)$ for the bending of the hydrogen bonds is represented by
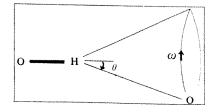
$$f(\theta) = C \sin\theta \, \exp(-s\theta^2/2kT) \tag{2}$$

where C is the normalisation factor, k is the Boltzmann constant, and T is temperature, taken to be 300 K. The factor, $\sin\theta$, is necessary because the number of possible configurations at a given value of θ is proportional to $\sin\theta$ and the energy dependence on ω is neglected.

The histogram for the distribution of the O–H $\cdots$ O hydrogen bond angles can be calculated using this procedure (Fig. 2).

Figure 2 also shows Kroon and Kanters's histogram[3] of 196 O–H $\cdots$ O hydrogen bond angles observed in molecular

Fig. 1 A bent hydrogen-bonded configuration.
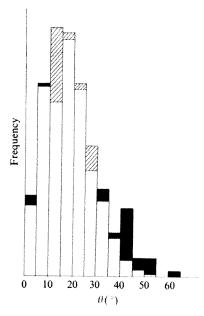
Fig. 2 The superimposed histograms of the theoretical and experimental distributions of O–H $\cdots$ O hydrogen bond angles. The experimental histogram is from Fig. 2 of ref. 3. Excess of experimental frequency over theoretical frequency is indicated by solid shading and excess of theoretical frequency over experimental frequency is indicated by hatching. Thus, open column indicates the amount of frequency common to both theory and experiment.

crystals. The two histograms agree quite well with each other: in our calculation the distribution shows a maximum at 14.5°, and the maximum from the molecular crystal data is approximately 15°. The slightly narrower distribution of our theoretical histogram compared with the experimental histogram may be because of the omission of the higher order terms in the right hand side of equation (1).

The hydrogen bond angle is a factor of the molecular structure and molecular arrangement in each crystal. The fact that the distribution of θ, found when the values of the hydrogen bond angle in various crystals are treated statistically, coincides with the distribution for an isolated hydrogen bonded structure, seems to suggest that the perturbation experienced by a structure of O–H $\cdots$ O in molecular crystals is very small.

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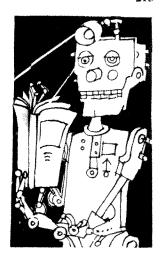
Marks of unknown carbonate-decomposing organelles in cyanophyte borings

BLUE-GREEN algae are a major agent of destruction of carbonate rocks and sediments, particularly in the littoral and shallow marine environments. The organisms discussed here are endolithic, that is, the individual algal filaments reside snugly in made-to-measure tunnels of their own, excavated chemically. Data presented here suggest that much of this geologically significant bioerosion is actually caused by what may be called digestion of the mineral substance, and is not

Book review supplement

IN spite of recent advances in the study of the history of education, many important tendencies and crucial decisions have never been fully explored. There is also a dearth of good biographies. The intelligent and industrious partnership of Ashby and Anderson has now provided, however, a lively and valuable contribution to this field. Their book is scholarly and compact and is based on a careful examination of a mass of scattered evidence.

Haldane was a front rank Liberal politician, more remembered for his army reforms than for his educational activities. Even his own autobiography has relatively little to say about education. Yet he was concerned throughout his life more with education than with any other topic and with the ends of education as much as with the means. He placed it in the forefront both of industrial progress and of democratic politics, and with a rare combination of psychological and political skills initiated or participated in many practical educational enquiries and enterprises. The very range of his activities and the extent of his commitments make his occasional failures as interesting and significant as his many successes, and the decisions which were not taken as a result of his advice or actions are as worthy of thoughtful investigation as the decisions which were.

Haldane believed profoundly in the unity and continuity of education—including adult education about which he had much to say. He also believed in a bigger place for science and technology within university institutions.

No one emphasised more than he the need for 'system' in education, yet he conceived of that system in organic and not in mechanical fashion : everything educational was part of the same "organic whole", from nursery schools to Workers Educational Association classes. It followed from this approach that universities were not separate

Visions of Haldane
Asa Briggs

institutions with their own vested interests but necessary elements—differentiated perhaps in styles—of 'national education', the development of which was successful "in proportion as it is guided by a policy which regards it in all its bearings and aims at settled objects". In practice, few educational institutions, except new ones, have ever approached national education quite in this way, and Haldane himself, who

chose his arguments to suit his audiences, would have been unsuccessful had he not been an indefatigable as well as a clever manipulator and, above all, "a great public personage".

Ashby and Anderson explain well how his approach, shaped by German philosophy and operated through well selected manoeuvres, affected the emergence or development of particular institutions — among them Imperial College and the University of Liverpool—and of the University Grants Committee as the guardian of the 'system'. Their conclusion is well balanced. They recognise that even without Haldane's personal contribution, much of what has subsequently happened in higher education in this country would have happened anyway. Yet without him, they add, "the style and tenor" of what has happened would have been different and "poorer". His conceptions were not original but through his own efforts they were "institutionalised". Of course, this only applies to some of them. When the Robbins Committee turned to structures in higher education years later, it referred to "a system if it can be called a system". The 'binary system' Haldane would have deplored—though all its outlines were there before he died—and there is still no 'system', even in an embryonic form, in adult education in spite of the valiant efforts not only of Haldane but also of Ashby.

It is possible to discern in the well organised pages of this biography sketches of alternative notions of education, deliberately less systematic or even non-systematic, for Haldane

always had his critics. He once wrote to Joseph Chamberlain mentioning "Oxford notions of what a University ought to be", and there would certainly have to be taken into account if this portrait of Haldane were to be expanded into a monograph on continuity and change in British higher education. The very idea of 'system' offended some critics, and the German philosophical language in which it was often expressed (sometimes with dangerous political potential) offended more.

Given a systematic approach in a country where it is easy to spurn enthusiasts in education as well as logic, what obviously counted then, as now, was the quality of the people making the key decisions concerning its operations and planning. Haldane was fortunate that there were far-sighted civil servants in London who shared his vision, but what would have happened had his ideas on 'regionalisation'— Ashby and Anderson call them a gospel —been fully implemented? Those are ideas which are not yet dead, although they are often advanced by people without vision or Hegelian philosophy —politicians and others seeking to coordinate in order to make education cheaper or "more equal". It would have been interesting had Ashby and Anderson pursued this story a little further, for they do not return to it in their epilogue. Haldane envisaged a system of 'regional' universities—and this involved bringing new universities into existence—each with responsibilities outside higher education as well as in extramural studies.

He never doubted, however, that the universities should "preside benignly" over the regional sub-systems which he called "educational provinces". The "great kind of coordination" of which

he dreamed was not achieved, but if it had been pushed further would it have been under the aegis of the universities? Haldane did not envisage the local authorities as the providers of funds—though he insisted that they should have an initiating and maintaining role in the dynamics of development —but even without funding powers would they have been willing to allow universities "the powers necessary to enable them to organise education from top to toe in their own districts"? How many of them, given expansion of student numbers, would have shared Haldane's view that higher education was "an end in itself"? How many members of his own party shared this view at the time?

Many of the most valuable paragraphs in this book are concerned with aspects of British higher education which do not relate exclusively to Haldane but which have never been very carefully considered in historical perspective. Thus, the sections on the University Grants Committee dispose of some historical misunderstandings and there are new insights into Fisher's Education Act of 1918. What often stands out is the meanness of the Treasury; a few thousand pounds can be argued about inordinately. It is sad to hear Haldane making remarks like "the only effective way of getting the money is by private appeals to very rich men", but it is sadder still to note that, leaving the Treasury on one side, very few rich men indeed showed the slightest interest in responding.

Portrait of Haldane at Work on Education. By Eric Ashby and Mary Anderson. Pp. xvi+202. (Macmillan : London and Basingstoke, November 1974.) £5.95.

To cross linguistic barriers

After Babel: Aspects of Language and Translation. By George Steiner. Pp. viii+507. (Oxford University Press: London, New York and Toronto, January 1975.) £8.00.

ONE strain in the extraordinary logical and philosophical ferment of the Thirties was I. A. Richards' thesis that literature itself had become a proper subject for scientific investigation in that general hypotheses about meaning and interpretation could be tested by the examination of texts. George Steiner's magnificent book should be seen as a survivor from that age of dinosaurs, still very much alive in our own time. To read the book one must actually be interested in literature; it is not enough to be schooled in any of the more recent attempts to handle language in a scientifically acceptable manner, such as Chomskyan linguistics or artificial intelligence.

In Steiner's view, the scientific approach to language has got into the hands of the bad guys, of people who have forgotten what language is actually like. To the extent to which he is right, the book should be compulsory reading for 'language scientists'. He draws attention to the fact that they are usually monolingual (he has been trilingual from an early age) and that little that they have to say is relevant to the real world of spoken and written language because they have a false picture in that they see language simply as a projection of whatever formalism they happen to subscribe to, a formalism that may be quite untrue to the language that comes out of their mouths, just as earlier theorists speculated about phlogiston while their lungs kept them alive by absorbing oxygen.

One might reply that criticism of a scientific activity by appeal to cultural information is absurd: Galileo's opposition, after all, had strong literary evidence for the movement of the Sun. But with language the case is different and, unlike Koestler, Steiner is taking on not established sciences, but would-be sciences like linguistics, philosophy and artificial intelligence; subjects on which Steiner is as much entitled to a hearing as the next man. In particular, he is arguing that anything claiming to be a theory of language must have something interesting to say about translation.

There are three obvious troubles with the way Steiner makes his case: his style of argument, the implausibility of his central thesis about the equivalence of translation and interpretation and the omission of a large issue that scientists would like to see raised (and

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THE brain is a mafs of foft matter, in part of a white colour, and generally ftriated ; in part of a grey or cineritious colour, having no fibrous appearance. It has grand divifions and fubdivifions : and as the forms exift before the folid bone inclofes the brain ; and as the diftinctions of parts are equally obfervable in anmals whofe brain is furrounded with fluid, they evidently are not accidental, but are a confequence of internal ftructure ; or ir other words they have a correfpondence with diftinctions in the ufes of the parts of the brain.

On examining the grand divifions of the brain we are forced to admit that there are four brains. For the brain is divided longitudinally by a deep fiffure ; and the line of diftinction can even be traced where the fides are united in fubftance. Whatever we ob-

serve on one fide has a correfponding part on the other ; and an exact refemblance and fymmetry is preferved in all the lateral divifions of the brain. And fo, if we take the proof of anatomy, we muft admit that as the nerves are double, and the organs of fenfe double, fo is the brain double; and every fenfation conveyed to the brain is conveyed to the two lateral parts ; and the operations performed muft be done in both lateral portions at the fame moment.

I fpeak of the lateral divifions of the brain being diftinct brains combined in function, in order the more ftrongly to mark the diftinction betwixt the anterior and pofterior grand divifions. Betwixt the lateral parts there is a ftrict refemblance in form and fubftance : each principal part is united by tranfverfe tracts of medullary matter ; and

The observations of Charles Bell. From *The Way In and The Way Out.* By Paul F. Cranefield. (Futura: New York, 1974.) $25.00.

almost every other issue one can think of is raised), namely scientific language. I shall look at these in turn.
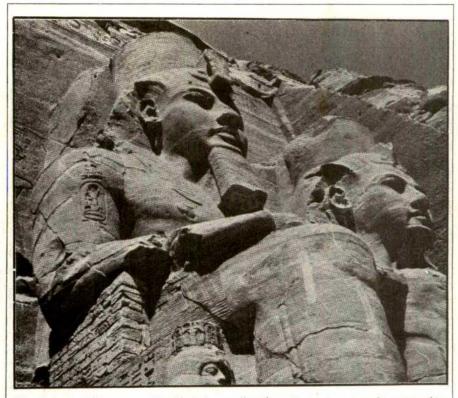
The central theme is the curse of Babel: the diversity of human languages; and the central thesis of the book is that every act of communication requires an interpretation, and that an interpretation is essentially a translation, whether across a language barrier, across a time barrier (as when we attempt to interpret Shakespeare) or across a barrier between individuals or groups. The book opens with a long speech from *Cymbeline*, and Steiner asks in detail what it means to us now. He turns to other passages in similar detail, ending with Noel Coward, always making the same point: a proper interpretation is hard, even at only 40 years' distance, but with the diligent application of critical method it can be done.

Before I confront his thesis briefly, a digression on the style of the book is essential. Under six chapter headings, Steiner raises an enormous number of issues, sometimes more than one on a page: does language determine our actual perception, as Whorf believed; what was Leibniz's view of a universal syntax and how does it relate to Chomsky's; is women's grammar different from men's; what effect had the statement of the second law of thermodynamics on sensibility and speech at large?

These topics are all raised and dropped during nimble and ebullient leaps from name to name and epigram to epigram: "History is a speech-act, a selective use of the past tense"; "Though the great master Tartakower thought otherwise, we do not ascribe feelings . . . to chess pieces"; "But for all their lively truth, children in the novels of James and Dostoevsky remain in large measure miniature adults. They exhibit the uncanny percipience of the 'aged' infant Christ in Flemish art. Mark Twain's transcriptions of the secret and public idiom of childhood penetrate much farther"; and even, "Sex is a profoundly semantic act".

Something intriguing is almost always being said, but it is seldom developed to a point of clarity, or to where the weight of literary evidence, familiar and arcane, that Steiner marshals can tell against his claims and throwaway remarks in any definite way. For there is no time to analyse and discuss in any depth as the whole vast parade of central European scholarship moves on, usually just at the point where one longs for detailed analysis and more examples.

Even what I called the central thesis is not discussed directly in the way it deserves, so let us return to that for a moment. The point of the thesis is to blur the distinction between interpreta-

The Colossi of Ramses at Abu Simbel, guarding the entrance to a temple penetrating 45 metres into solid rock. Possible evidence that the now barren Nubian desert was once comparatively fertile. From *The Ecology of Oases*. By J. L. Cloudsley-Thompson. Pp. vi + 43. (Merrow: Watford, 1974.) £1.25; $4.40.

tion and translation: between the way an individual confronts his own language and the way different languages confront one another. Steiner gives too much weight to what one might call the Telex view of human understanding: that we sit, as it were, in our private offices trying to interpret language coming in from outside. This view makes human communication seem a problem, and is a recrudescence of an old-fashioned empiricism, one that ignores the essentially performative and social nature of language.

If one rejects the 'Telex view' there seems an essential difference between trying to understand what another speaker of your own language is saying, and doing the same for a different language. In the case of interpretation we have the ability to go on asking someone what he means, to form hypotheses, as it were, and test them in discussion. In the case of translation that is exactly what we do not have, and thus the distinctive problems of translation arise. We are, in general, deprived of just that essential "further elucidation" aspect of language.

Steiner's key examples are of interpreting one's language diachronically: he argues that any English of the past is another language, and similarly for the language of women, of the upper classes, and so on. The heart of the matter is the elusive Wittgensteinian notion of a "form of life": in that we can interpret/translate only if we share
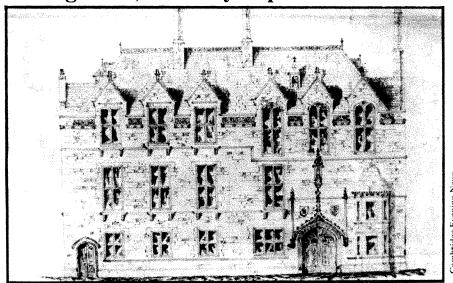
an adequately close form of life with those who are the source of the language. One might say dreamily that it is this background knowledge that workers in artificial intelligence are at present trying to formalise and put into their programs.

A large topic presents itself here that it would have been nice to see Steiner discuss: namely, is science such a form of life, and if so can scientific language transcend other cultural boundaries? The question should have come up in the extended discussion of Whorf and his thesis that different cultures have languages describing essentially different worlds. Steiner might well have brought out that Whorf explicitly extended this thesis to scientific language and argued that a psychologist, physiologist and physicist describing, say, the same brain, are speaking different, not mutually translatable, languages and, in that sense, have different cultures. This seems implausible, for we feel that science is a uniform international culture and language. And yet, had Steiner found room for this topic he would almost certainly have drawn our attention to modern Chinese scientific journals, particularly medical ones, where it is not at all clear that they can be understood outside the Maoist frame of thought.

It is a highly personal, stimulating and infuriating work but, warts and all, a considerable achievement.

Yorick Wilks

Solid ground, Himalayan peaks

The Cavendish Laboratory, 1874–1974. By J. G. Crowther. Pp. xvi+464. (Macmillan: London and Basingstoke, October 1974.) £25.00.

THE foundation of the Cavendish Laboratory a century ago was the greatest single consequence of the mid-Victorian movement to advance the cause of science in Britain. During the preceding two centuries the development of science in the English universities had been paralysed by the Clarendon Code which, as G. M. Trevelyan said, subjected them to "the bondage of Church monopoly" so that only professing Anglicans could teach and study there. Most science in England was therefore found outside the universities—in the Lunar Society at Birmingham, or the Literary and Philosophical Society at Manchester, or the Royal Institution—a fact from which Cardinal Newman ingeniously argued that experience had shown that for its own well-being science should be pursued outside universities rather than within them. Only in Scotland, where the Clarendon Code had been repealed following the 1688 Revolution was it possible for non-Anglicans and free thinkers to go to university—a fact which contributed much to the eminence of Scottish science, philosophy, and economics in the century and a half before 1871, when at last the University Test Act abolished religious discrimination in England.

In the same year James Clerk Maxwell was appointed as the first Professor of Experimental Physics in Cambridge. The university had procrastinated for some years over the creation of the Chair and its associated laboratory, having been influenced by such remarkable arguments as "A Prussian is a Prussian and an Englishman an Englishman and God forbid that it

should be otherwise" by the Master of Sidney Sussex. What finally moved the university was the offer (which was accepted) by its Chancellor, the Duke of Devonshire, to cover the cost of building the laboratory and of buying its equipment.

In this history, Mr Crowther sets the foundation of the laboratory in perspective, and takes us through its continuously happy fortunes ever since. No Chair in the world has had such a distinguished and creative a succession of holders, and Mr Crowther provides many sidelights on their personalities. We find Maxwell, for example, working in his shirtsleeves with each hand in a basin of water to compare the strengths of electric currents, and exclaiming "each man his own galvanometer!" by way of explanation to an American visitor, S. P. Langley (of the bolometer and flying machine), who had been brought unexpectedly by Schuster to be introduced.

Maxwell at the time was confirming the experiments of Henry Cavendish, whose papers he edited in what Mr Crowther describes as "the finest contribution to the history of science in the English language". Maxwell had a strong historical sense but Mr Crowther makes the general point that "The Cavendish, like the whole of British science, was sublimely disinterested in its historical aspects. Why British science should have been so non-historical is a significant question". Perhaps it is a sign of exhilarating progress to have so much to do and think about in the present and the future that effort in attending to the past, or even preserving the present for the future, is grudged. But it has resulted in a cleavage in Britain between practising scientists and those who style themselves "professional historians of science" that too few of us are trying

to repair, and it is a pleasure to acknowledge Mr Crowther's splendid efforts over many years in this direction.

Among the many illuminating comments that he has gathered from past and present Cavendish members there is one that—if it really means what it says—is mildly surprising: "Some consider that the Cambridge undergraduate . . . has done more for Cavendish science than the talented research students coming from outside. They produced a large body of very good scientists of the second level, which provided an excellent support for the front rank of genius". Such a comment must have overlooked the fact that J. J. Thomson, Rutherford, Chadwick, Aston, C. T. R. Wilson, Cockcroft, Ryle, and Crick were all undergraduates elsewhere; and Maxwell took his first degree at Edinburgh.

Besides its value as straightforward history there is much to be dug out of the book by anyone interested in the problems of running a laboratory and of seeing it through from one phase of success to another. There is, for example, the principle that Bragg took over from military organisation that one man cannot effectively keep contact with more than six subordinates on a day-to-day basis, which led to the organisation of the large laboratory into groups which in turn may have again to be sub-divided. And those who press for the centralisation of university services such as workshops might be reminded of the experience that, even inside a single laboratory such as the Cavendish, "It was much more economical to do as much as possible in a small workshop belonging to a group rather than in a central workshop". And again there is Bragg's estimate that Britain produced one good physicist a year per million inhabitants—a sobering reflection on the optimism of the Robbins expansion.

The one truly unfortunate thing about the book is its price—£25.00 after the publishers had already advertised it at the prohibitive price of £14.00. And especially in view of the price the quality of the photographic reproductions is poor. For neither of these defects is Mr Crowther responsible, and it is much to be hoped that somehow the story which he tells will be made much more widely available than the present price allows. For the story is magnificent: Maxwell and Rayleigh setting the foundations; J.J. discovering the electron (when the total bill for 1896 was £1,067 7s 0d plus his own salary and another £420 for the salaries of university lecturers and demonstrators); C.T.R. inventing the Wilson Chamber; Aston finding the mass defect and thereby the clue to nuclear energy; Cockcroft and

Walton transmuting lithium with protons; Chadwick finding the neutron; Blackett recognising the positive electron; Crick and Watson elucidating the structure of DNA; Josephson predicting his effect; Hewish and Jocelyn Bell finding pulsars. Besides this Himalayan chain of peaks of discovery in the Cavendish itself there have been major discoveries by Cavendish men working elsewhere—for example, the wave nature of the electron by G. P. Thomson or the π-meson by C. F. Powell. But the Cavendish story is not just of these peaks; there has been an enormous amount of supporting work over a wide range of physics, and from the Cavendish many physicists have gone outside to other universities, to the schools, to industry, and to government establishments.

So many achievements could induce complacency; but so long as the Laboratory maintains the spirit of Professor Pippard's 'The Cavendish Tradition' (*Nature*, **249**, 602–603; 1974) it need have no fear that "Nothing fails like success".   **R. V. Jones**

Scientists only human in their quest

Originality and Competition in Science: A Study of the British High Eenergy Physics Community. By Jerry Gaston. Pp. xix+210. (University of Chicago Press: Chicago and London, 1974.) £5.50.

GASTON'S sociological study of the British high energy physics (HEP) community is most interesting. Knowledge in the natural sciences with all its fallibilities is less uncertain and less subjective than knowledge in the social sciences or the humanities. The corollary has, too often, been a belief that natural scientists conduct their scholarly inquiry in a more rarefied way than other people and are indeed rather more rarefied people. This belief has been encouraged by the historians and philosophers of science and sociologists have added their norms. Dr Gaston quotes four norms or rules which Robert Merton ascribes to scientific activity: organised scepticism, universalism, communality and disin-

terestedness. Each norm is not unique to science but together they are said to be peculiar to the ethos of modern science. American sociologists of science, led by Merton himself and by scholars such as Hagstrom have, however, shown how often scientific activity diverges from those norms.

Dr Gaston looks at some of the norms in relation to the British HEP community at one point in time—1967–68. One of the advantages of his study is that its coverage is almost complete and does not rely on small samples: about 220 scientists were located at 23 different universities or government establishments, of whom 203 provided information and were interviewed.

The author has analysed their social, educational and professional background; the kinds of research they conducted; their contributions to the scientific community—that is, their productivity; the recognition they received from the scientific community; their attitudes towards research; the prevalence and severity of competition; and their communicative behaviour. Throughout the book, in pursuing these themes, Gaston distinguishes between the views and habits of theoretical and experimental physicists.

He also makes valuable comparisons with the studies of American scientific communities. It is, however, unfortunate that he follows too closely the pathways set by his predecessors so that some of the problems which are especially interesting in HEP are omitted. Thus, he omits the scientists who operate accelerators and other machines "because they are more akin to engineers than scientists". He does not mention the machine builders at all, but it is wrong to dismiss the machine-providers whose performance in terms of sheer physics alone has been so great. The relationship between them and the machine-users is important in the study of any HEP community. Again, in high energy physics, the identity of the individual scientist has increasingly been lost within team identity. Much more attention needs to be given to the effects of this change in the property system of science upon motivation and relationships.

These qualifications apart, Gaston produces 45 statistical tables which reveal fascinating positive or negative correlations. For example, why are high energy physicists significantly more productive at some institutions than at others? How is the number of publications related to prestige of the university at which the PhD was gained, prestige of present affiliation,

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The Ciba Foundation: An Analytic History, 1949–1974. By F. Peter Woodford. Pp. viii+212+8 plates. (Associated Scientific : Amsterdam, Oxford and New York, 1974.) Dfl. 38; $14.75.

THE decision of the Ciba Foundation to commission an analytical history of its first 25 years has turned out to be a wise venture. The analysis, undertaken by Dr Peter Woodford, has been carefully performed and, through his avowed aim to be critical, he has managed to bring out a number of points of importance. The Ciba Foundation with its symposia and publications is so well known that it is in danger of moving beyond the range of criticism. It is, therefore, of importance to analyse the secret of its success and to try and separate the method from the qualities of those who run it. Inevitably, that is an impossible task.

The Ciba Foundation is a product of the knowledge, experience and attitudes of Dr Gordon Wolstenholme and his able colleagues. The fact that their purpose and method of working was timely and has filled a special place in the world of medical research is a further tribute. Maybe all our institutions are a product of individual effort at a special time. Certainly, it is always very difficult for any successor to take over and continue where his predecessor left off and this will be equally true of the Ciba Foundation because of the unique, accumulated interpersonal relationships developed by its staff.

The importance of this book lies, therefore, in its analysis of those features of the foundation that may be greater than its leaders. The analysis of the reasoning which led to the decisions on the size and nature of the meetings in general and also for more specific topics is thus the most important aspect of the book.

It is also important that a study has been made of the impact on medical research of the activities of the foundation. This has proved to be a difficult task which has daunted many who have previously attempted it in other organisations. Sufficient to say that the record of the Ciba illustrates the flow of progress that can develop from bringing toegther, in relaxed surroundings, people from different disciplines and nations so that they may stop for a while to contemplate matter that are set on one side in the busy working day. The Ciba Foundation has achieved its aim and provides the ideal illustration of the importance of having specialised activities supported by private funds. It also shows that it is not size, but quality, that counts.

I can make only one factual criticism. It is not correct to say (page 4), "The essence of the law (Charities Act) is that those who provide the money disposed of by the trust may not be represented on its governing body". This book will be of interest to all those who know and admire the Ciba Foundation through its books or meetings. It is a worthy tribute to the singleness of purpose of Gordon Wolstenholme.   **P. O. Williams**

proportion of time spent on research, number of hours spent weekly on research, proportion of time spent on teaching, class of undergraduate degree, chronological age, professional age? What is the relationship between recognition and these variables and what is the relationship between productivity and recognition? Competition is measured by the number of times scientists have been anticipated through having research findings similar to their own published first by someone else. What anticipation have British HEP workers experienced, for example in relation to their rank or by comparison with American scientists? Having been anticipated, did they publish or not publish? As for communication, what is the most important method of obtaining information relevant to research, by type of HEP, rank, professional age?

How does verbal communication work and which are the most important journals?

Equally, if not more, illuminating are the extracts from transcripts of conversations about competition. One suggestion is that competition helps to motivate scientists who otherwise might put forward less than their best effort. An experimentalist is quoted as saying, "I have suspicions that less than one physicist in three that I've met is really and deeply and drastically motivated by a desire to 'find out' ". There is much material in this book to show just how cut-throat the competition can become —both between individuals and between continents—together with material which suggests why American physicists 'beat' European physicists in certain races of discovery. Gaston records his evidence about the "three main

methods of deviance" which scientists sometimes use to get the recognition which eludes them: hasty publication; fraud and theft; and secrecy.

In his conclusions, the author does not ask squarely where his own evidence leaves Merton's four rules of science. He finds that the reward system in British HEP communities, unlike the system in America, operates in a universalistic fashion. Recognition is highly related to productivity and theorists receive more recognition than experimentalists. But affiliation with a particular type of university or a department in a particular prestige category has no effect on opportunities for scientific recognition. The four rules are, however, meant to hang together and Gaston shows that scientists are indeed human in their quest.

Margaret Gowing

The Competent Infant: Research and Commentary. Edited by Joseph L. Stone, Henrietta T. Smith and Lois B. Murphy. Pp. viii + 1,314. (Tavistock : London, 1974.) £11.00.

MOST books consisting of collections of published papers are worth neither reviewing, buying nor reading: the present volume is an exception. The editors have done a remarkable job of arranging the selected papers in logical order, shortening them, and with the help of their own commentaries producing a coherent book. There are two further reasons why this collection is particularly useful. First, infant development is a topic on which spectacular progress has recently been made and second, as there are few journals devoted specifically to it, papers on the subject have appeared in such a wide range of journals that no one person could possibly scan them all: in *The Competent Infant*, we find papers from the *Journal of Ophthalmology* rubbing shoulders with extracts from *Psychosomatic Medicine, Vita Humana,* and *Cognitive Psychology.*

The book demonstrates the ways in which our ideas about the competence of infants have been revised drastically by the work of such pioneers as Wolff, Escalona and Prechtl. Until some 20 years ago, it was widely accepted that new-born babies were almost entirely passive and incapable of anything much in the way of organised adaptive behaviour. More recent research shows quite the contrary and the watershed may be placed at the publication of Wolff's now classic paper in 1959. He found that, if you catch your infant at the right moment, it will emit highly organised and complex behaviour. As Prechtl puts it: "If you put a new-born infant baby in a supine position

in its cot and cover it with a blanket up to the neck, of course it gives the impression of being a kind of vegetable which just cries and sucks from time to time and that's all". What Wolff discovered was that the reactivity of the new born depends on its state. He classified such states into sleep (regular, periodic and irregular), drowsiness, alert acvtivity and alert inactivity. It is only when the infant is alert and

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More than vegetables

inactive that it will attend to outside stimuli; when it is alert and active, crying or threshing around, it pays little attention to external stimuli, particularly to new stimuli. During the first week of life, the infant spends only about 10% of the day time in alert inactivity. In this state "his respirations are regular . . . his eyes are wide open, shiny and capable of conjugate eye movements, and he makes visual and auditory pursuit movements . . . the skin is pink but not flushed". Such

behaviour tends to occur immediately after the baby has satisfied its physiological needs such as feeding and defecating. Experimenters patient enough to wait for such periods have been rewarded by observing complex behaviour even on the first day of life: some infants in fact show about an hour of alert inactivity starting 15 minutes after birth.

Recent research, stemming from Wolff's insights, has shown that there is considerable innate organisation of perceptual and motor functions. One of the most remarkable experiments republished in the present book is Sackett's ingenious demonstration that monkeys have an innate capacity to recognise not merely other monkeys but whether or not the other monkey is in a threat posture; this work is now nearly 10 years old and its implications are so important that it is very regrettable that, as far as I know, there have been no independent attempts to replicate it. There is also ample evidence that personality traits which tend to remain constant throughout life may appear in new-born infants: American babies of Chinese origin show less irritability and impatience than do whites born on the same ward and tested within a few hours of birth.

The book should give pause to those who think either that psychologists rarely discover anything we did not know already or that introspection and every day experiece are all we need use to infer the structure of the mind— a belief that has recently been resuscitated by some polemicists for the new branch of technology known as 'artificial intelligence'.

The book is 1,300 pages long and nicely produced: at £11.00 it surely rates as a best buy. **N. S. Sutherland**

More behaviourism

About Behaviourism. By B. F. Skinner. Pp. 256+viii. (Jonathan Cape: London, February 1975.) £3.50.

PROFESSOR Skinner is unrepentant. Assailed by the liberal and radical establishment as a reactionary witch-doctor intent on subjecting human freedom to blind conditioning, attacked by the new school of linguistics and cognitive epistemology as an ignorant primitivist, Skinner not only maintains his position but makes it more dogmatic.

The concept of mind is an obscurantist 'invention' largely attributable to Plato. The only distinctive characteristic of verbal behaviour is the fact that "it is reinforced by its effects on people". Dreams, fantasies, *déjà-vu* are merely re-cognitions of "what we have once cognised", in some of which certain controlling features of self-knowledge are defective. Human thought equals human behaviour: *sum ergo cogito*. Faith is just "a matter of the strength of behaviour resulting from contingencies which have not been analysed". Mentalist explanations "explain nothing". Behaviourism, on the contrary, puts us in a position to alter and improve the condition of mankind if only we will apply its commanding insights into the role of the environment and into the agency of such cardinal notions as reinforcement and operant behaviour.

Neither the strategy of the argument nor the tone of mildly outraged common sense are at all new. If there is innovation it can be found in the tactics of Professor Skinner's case, particularly in his increasing resort to a Darwinian idiom. In an important sense, we are told, "all behaviour is inherited, since the organism that behaves is the product of natural selection. Operant conditioning is as much a part of the genetic endowment as digestion or gestation". The origin of behaviour "is not unlike the origin of species. New combinations of stimuli appear in new settings, and responses which describe them may never have been made by the speaker before, or heard or read by him in the speech of others. There are many behavioural processes generating 'mutations', which are then subject to the selective action of contingencies of reinforcement."

What is going on here is plain. Darwinian and post-Darwinian terminology is being used in an attempt to refute the drastic onslaught on behaviourism by Chomsky (whom Skinner, either in coyness or contempt refuses even to mention by name). Dismissing the entire basis of innateness in transformational–generative theories of speech and consciousness, Skinner declares that "grammatical behaviour" has always been shaped by the reinforcing practices of given verbal communities in which some behaviours were more effective, more provocative of useful response, than others. Sentences are not generated by imaginary 'deep structures' but, like all other human proceedings, by "the joint action of past reinforcements and current settings".

As one follows this controversy, a sense of exasperation mounts. It is so utterly clear that both sides simplify, distort, and finally trivialise reality. Skinner is perfectly right when he observes that Chomsky's model is full of logical and evidential loopholes, that it absurdly neglects environmental-historical factors, that it is dogmatic and even 'mystical' in its presupposition of deep structures and mental 'presettings'. Chomsky, on the other hand, has shown beyond doubt that a stimulus–response paradigm along Skinnerian lines is totally inadequate to explain either the process of language acquisition and innovation or the prevalence (or what looks like the prevalence) of rule-governed similarities across the field of all known grammars. But, surely, there is no need here of a doctrinal either/or. The evolution of human speech and cognition is neither Skinnerian nor Chomskyan, but represents a constant dynamic reciprocity between universal neurophysiological features on the one hand and the contingencies of the environment on the other, between predisposition and reinforcement, between mimesis and discovery. Indeed, using a Darwinian analogue, one would want to argue, with far more attention to the facts than is shown by either behaviourism or transformational–generative linguistics, that man's language capacity is uniquely adaptive. Through words he constructs worlds more bearable than that of his organic surrounding. Through the future tense he does something to circumvent the totality of biological death.

Skinner's contributions to experimental psychology, to learning theory and the understanding of conditioning and reinforcements stand secure. It is a pity that they should be overshadowed by an embattled dogmatism the political add philosophical consequences of which are not necessarily sinister, but are certainly naive.   **George Steiner**

Animal Nature and Human Nature. By W. H. Thorpe. Pp. xviii+435. (Methuen: London, January 1975.) £7.20.

IT is of interest to compare *Animal Nature and Human Nature* with Skinner's new book, *About Behaviourism*. Although Thorpe thinks human behaviour cannot be explained solely by genetic and experiental factors whereas Skinner adopts a strictly deterministic position, the similarities between the two books are more striking than the differences. Both authors believe that if human behaviour were so determined, our freedom of will would be constrained; both rely heavily on assertion and on appeals to authority rather than on argument, though naturally the quoted authorities differ; both dogmatically reject computer programs as possible models of the mind without examining any such programs; both put forward Utopian views of man's future without taking the trouble to think out what sort of Utopia they want; neither gives evidence of having read, let alone

All slots open, flaps down, and undercarriage lowered, the barn owl approaches home. From *Natural History Photography.* Edited by D. M. Turner Ettlinger. Pp. xxvii+395. (Academic: London, 1974.) £8.80; $23.25.

understood, any arguments against their own position; finally, nothing of any scientific or ethical consequence follows from the contrasting stances taken up by each author.

Thorpe's book is perhaps the less bad, if only because, unlike Skinner, he gives some interesting examples of the results of his own science. He culls from recent work in ethology instances of fixed action patterns, animal communication systems, and social organisation in mammals. Such phenomena have an inherent fascination: like most ethologists Thorpe is at his best when describing such behaviour and speculating on its survival value. He is less good at specifying the mechanisms that mediate it. On many issues, such as nature against nurture, he takes a sensible position—he rightly castigates Hebb for his declaration that it is absurd to ask how far a given piece of behaviour is innate, how far it is learned. Thorpe's chapter on perception is weak: he still thinks of perception in terms of pattern recognition and fails to get across the idea that perception must involve forming rerpesentations of the outside world which can be manipulated in ways that serve to guide the organism around. In this chapter, his accounts of much experimental work are somewhat cursory and inaccurate.

In arguing for an antireductionist, emergent view of mind he fields the standard team of obscurantists: Koestler, Polyani, McKay, Eccles, Dobzhansky, Weiss, Hardy and Teilhard de Chardin. His eleven is made up by enrolling two new players, Chomsky and Popper, and by using a theologian (Hick) as long-stop. With the exception of the two beginners, these players proceed to throw around terms like 'emergent', 'consciousness', 'determined', 'free-will', 'wholes-greater-than-the-sum-of-their-parts', and so on. We are treated to such sentences as "the ego's view of the physical world will thus be mediated by a mechanism working just like television". On page 341 there is even a diagram showing (with a dotted line) how "mental space" intersects three-dimensional physical space. The players have such an enjoyable time tossing the ball to one another and cheering each other on, that they seem quite to have forgotten to notice the opposition. Nevertheless, they make their opponents' task very difficult, since the game is played with no rules or definitions; but it may be worth examining a few of the many *non sequiturs.*

Thorpe accepts McKay's proof that behaviour is indeterminate: in attempting to predict our own behaviour, we change our internal state and, short of an infinite regress, we cannot take such changes into account in making our

predictions. The premise is correct but the conclusion does not follow: do we regard computers as being indeferminate because no program could completely predict what it will do? Again, it is correctly stated, following Popper, that at each level of explanation it is necessary to evolve concepts appropriate to that level. To explain and predict the behaviour of a gas we need concepts like pressure, appropriate to collections of molecules not to individual molecules, and to explain the workings of a computer program we need concepts such as 'conditional jump', 'recursion', and 'iterative loop' that cannot be applied to transistors. If this is all Thorpe means by "emergent qualities", then we can readily concede that a system with the highly unusual organisation of the brain is likely to have emergent qualities, without committing ourselves to the belief that there is some special sense in which human behaviour is 'undetermined' and without supposing that there is a two-way interaction between consciousness and matter. Thorpe also uses the "gawping at Nature" argument: we do not understand why some bird songs and displays should be as elaborate as they are, but present ignorance is no reason for assuming that no scientific explanation will in future be found. It is particularly curious that Thorpe should use this argument since else-

where he points out that some highly complex behaviour once thought to indicate the presence of a life-force or creator can nowadays be explained in mechanistic terms.

The asertion that "Sir Karl Popper comes down with all the weight of his great learning and experience heavily on the side of dualism" surely does not exempt Thorpe from considering the difficulties inherent in this position. The polemical parts of the book are written in the vaguest of terms. Thorpe dismisses computers as models of the mind without discussing any work in artificial intelligence, yet telepathy and clairvoyance are uncritically accepted on the strength of the questionable work of such experimenters as Soal and of Koestler's collection of unlikely coincidences. The distinguishing characteristic of the human race is said to be religion.

The book is based on a series of Gifford lectures delivered at St Andrews: such lecturers are instructed, under Lord Gifford's will, to deal with natural religion "as a strictly natural science, the greatest of all sciences"; but this instruction is usually interpreted by the lecturers themselves to mean they should treat of their own subject matter in religious terms. The present book is a fine example of the Gifford Lectureship Syndrome.

N. S. Sutherland

Factors leading to schizophrenia

Genetics, Environment and Psychopathology. (North Holland Research Series on Early Detection and Prevention of Behaviour Disorders, vol. 1.) Edited by S. A. Mednick, F. Schulsinger, J. Higgins and B. Bell. Pp. xiv + 346. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1974.) £9.25.

IN 1962 the Psykologisk Institut in Copenhagen began a research project aimed at the early detection and prevention of mental illness. This book brings together a number of studies conducted at the Institute over the last 10 years. Many have contributed to the research, and 20 authors from the USA, Denmark, Iran and the UK, write in this volume.

The authors were dissatisfied with research on people already schizophrenic and so they conducted prospective studies of people at risk. They used children of schizophrenic parents and selected control groups. Unexpectedly, they found that perinatal factors influenced later outcome and so they established a new study in greater detail, using babies born in Copenhagen between 1959 and 1961. They linked that with Professor Preben Plum's detailed peri-

natal study of 9,006 deliveries during that period.

The methods that were used are described fully. They were of a high standard and good contact was maintained with family doctors and hospitals. There is a full list of references which will be valuable to others studying in this field, and there is an index.

The book represents a considerable advance towards the unravelling of genetic and environmental factors in the origins of schizophrenia. Electrodermal responses were used in several of the studies, and some of the authors characterise schizophrenia as a learned pattern of avoidance responses. As treatment seems to offer little, attention has been concentrated on methods of prevention of the development of schizophrenia in 'high risk' children. The authors have made excellent use of the Danish Register of Adopted Children. Schizophrenia is only one of the mental illnesses studied, and the effect on being reared by a schizophrenic mother is tentatively separated from inherited factors.

This volume is good value for money for all research workers in this field and will be of great interest to all field workers. **R. Mac Keith**

Evolution of sex

THIS is an important and infuriating book*. It is important because it is a scholarly and sustained attack on the most important problems in evolutionary biology; the evolution of genetic mechanisms, of sexual dimorphism and of society. It is infuriating because it fails, narrowly but decisively, to provide the illumination promised by the first two chapters.

The problem which Ghiselin sets out to cover is more easily stated than solved. In Darwin's theory of natural selection we have an explanation of evolution in terms of the success or failure of individuals to survive and leave descendants. As an explanation of characteristics which promote individual survival and reproduction, this is fully satisfactory. But sex and genetic recombination are often interpreted in terms of their contribution to the survival of the species and not of the individual, and social behaviour in terms of the survival of the community. How is the evolution of such characteristics to be explained? Two positions seem possible. The first, the "group selection" explanation, is to argue that selection between species (that is, the survival and division of some species and the extinction of others) produces genetic mechanisms adapted to ensure species survival. The second, strongly espoused by Ghiselin, is that sexual adaptations evolve because of the advantages they give to the survival and reproduction of individuals—that is, by natural selection in Darwin's sense.

Ghiselin has qualities which adapt him for tackling these questions. His philosophical position is sound (that is, I agree with it); he distrusts teleological explanation, despises Baconian induction, and regards holism as an illegitimate attempt to smuggle teleology back into biology. He has a sensitive nose for Panglossianism, which can be defined as the belief that the characteristics of organisms exist to ensure the survival of species and of ecological communities. Although accusations of Panglossianism are usually indignantly denied, such views are widespread among evolutionists, ecologists and ethologists, as Ghiselin makes clear. Equally important, he has the widespread knowledge of natural history which is needed to test evolutionary hypotheses.

Why then does he fail? Ultimately, because he does not think as a geneticist, and because he abjures mathematical language. He rightly remarks that if a mathematical model fails to

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*The Economy of Nature and the Evolution of Sex. By Michael T. Ghiselin. Pp. xii+346. (University of California: Berkeley, Los Angeles and London, December 1974.) $12.05; £7.10.

Understanding Homosexuality: Its Biological and Psychological Bases. Edited by J. A. Loraine. Pp. 217. (Medical and Technical Publishing: Lancaster, October 1974.) £6.50.

HOMOSEXUAL behaviour is of major interest both scientifically—because of the general problems of the sources of sexual preference, gender identity and gender behaviour—and socially, because of the legal, occupational and other disadvantages suffered by those whose preference is homosexual. Three of the chapters in this book fall under the scientific heading and five under the social heading; the remaining chapter is concerned with venereal disease and homosexuality.

Two of the scientific chapters are reviews, one mainly on males, by Cooper, and one solely on females, by Kenyon. Both are reasonably adequate on the biological sources of homosexual behaviour—Kenyon particularly so—and on parent–child relationships, but neither pays more than passing attention to the potential contribution of the psychology of learning to the acquisition and maintenance of homosexual behaviour. Classical and instrumental paradigms and direct as well as modelled experiences are likely to be relevant—as they are for all kinds of behaviour. Freund *et al.* report an interesting series of experiments which sought, but did not find, evidence for bisexuality of response. In all three scientific chapters almost all reported studies compared heterosexuals with undifferentiated homosexuals, rather than using the heuristically more useful division into primary (life long) and secondary (non life long) homosexuals.

The remaining chapters are concerned with the legal, theological, and general social responses of the heterosexual majority to the homosexual minority. Grey contributes a particularly thoughtful, sane and eloquent plea for the equality of status of all sexual variations involving consenting partners, both as objects of scientific study and in terms of social esteem. Ramsay and others describe the rapid progress towards equal status in Holland. A final chapter, on the status of the homosexual, by Chew *et al.*, skims rapidly over both social and scientific aspects and includes the provocative assertion that heterosexuality is the "primary cause" of the "dilemma of overpopulation".   **Philip Feldman**

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predict the nature of reality, it is the model which must be changed. But models do make clearer what is being assumed (even if tacit or unconscious assumptions are made, as is often the case, a careful examination of the model will usually reveal them), and they show more certainly whether the conclusions follow from the assumptions. Ghiselin seems to me to fall down on both these points. For example, on page 69 he offers an explanation for the association between diploidy and complex multicellular structure. I simply cannot form a clear picture of the genetic and selective mechanisms being proposed.
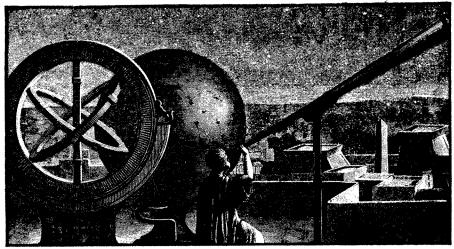
On the still more crucial point of the short-term individual advantage of sex, my difficulty is different. I do understand, in a very vague way it is true, what is being proposed. The snag is that I am familiar with several attempts to make these proposals more precise. It turns out that the conclusion—an immediate advantage for sex—seems to follow only if one makes rather extreme and implausible assumptions. Ghiselin would presumably retort: so much the worse for the model. Oddly enough, I agree with him. Plausible models giving an individual advantage to sex may well be possible; Williams and Mitton have made a promising start. But it simply does not do to ignore the difficulty and to appeal to nature as a witness. If we do not have a clearly formulated theory we do not know which facts support it.

Ghiselin's reluctance to think genetically lets him down in other contexts—for example, in his discussions of the role of kin selection in the evolution of societies, and of Fisher's idea of a positive feedback on female choice in sexual selection (it is uncharacteristic that he does not ascribe that idea to Fisher; one of his virtues is his respect for history). Thus, he seems to regard kin selection as a needless complication, only to be invoked if nothing else will do. I would argue that it is a process which is bound to operate whenever relatives live close to one another, and which therefore must be considered in any discussion of the evolution of societies. I suspect Ghiselin would dismiss this as *a priorism*; in fact, I am asserting that if we accept the (empirically tested) theories of genetics, then certain things follow, among them Fisher's and Hamilton's arguments. Of course, the relative importance of these and other processes have to be investigated in particular cases.

There is much in the book that is stimulating and illuminating, particularly in the discussion of sexual selection. But in some ways Ghiselin invites an ungenerous review. He is curiously dismissive of other people's ideas, with the exception of Darwin's. It is true that Panglossianism is rife, but it is not universal. Other evolutionists have been attempting non-teleological explanations of these phenomena. Ghiselin may have more allies than he thinks.   **J. Maynard Smith**

Covering the Universe

Astrophysics of Gaseous Nebulae. By Donald E. Osterbrock. Pp. xiv+251. (Freeman : San Francisco and Reading, January 1975.) £8.90.

IN the Milky Way and in other galaxies the very brightest stars are generally surrounded by masses of bright interstellar gas. These are the H II regions, so called because the hydrogen there is in the form of H^+. Other atomic species, notably helium, are also largely ionised as well.

All of these ions are continually recombining with the electrons present. If recombination takes place to an excited state then the resultant downward cascade of the recombined atom or ion produces a rich spectrum of emission lines. The optical observation of H II regions relies on these lines; the radio observer can also detect spectral lines arising from transitions between very high levels of the atom concerned. H II regions show up in the radio continuum as well. The emission process here is free–free emission by an electron in the field of an ion.

In principle, the observations contain an enormous wealth of information about the density, temperature, and composition of the gas in H II regions, and its distribution in space. But to tap this wealth requires a considerable amount of physical analysis. Much of the required work has been done by Don Osterbrock and his colleagues; in this book he presents an account of the subject which is so good that it should be unnecessary from now on to consult any earlier work.

The processes by which an equilibrium is attained in a diffuse mass of gas sitting around a bright star which is rich in ultraviolet radiation include radiative transfer and electron collision processes; since many different types of atom are involved the situation becomes quite complicated, but the author never lets it get out of hand. For good measure he also deals with planetary nebulae, where many of the same phenomena occur on a smaller scale.

But there have been many developments in our understanding of H II regions since the text was completed (about three years ago) and in particular much attention is now being focused on compact H II regions. These are objects whose linear dimensions are smaller. They are often heavily obscured by dust, and are therefore observed mainly at radio and infrared wavelengths. There is a definite association between dense molecular clouds and these compact objects. Very probably they occur in regions where star formation is still active, and the stars involved in them must be among the youngest objects in the sky. Some of these developments are hinted at in the book, but I hope that Professor Osterbrock will be persuaded to write a second, more detailed, volume to deal with these more recent discoveries.

F. D. Kahn

Interstellar Communication: Scientific Perspectives. By Cyril Ponnamperuma and A. G. W. Cameron. Pp. 226. (Houghton Mifflin: Boston, 1974.) £3.50.

ONLY two serious possibilities exist for acquiring information about extraterrestrial life. The first is by direct rocket flight within the Solar System. It is possible, though by no means sure, that one of our sister planets may possess a primitive biosphere. The second is by interstellar communication with remote technical communities elsewhere in the Galaxy. With the declining interest in the spaceflight programme, the exciting if somewhat exotic, possibility of radio communication with extraterrestrial civilisations is achieving mounting respectability and being given serious consideration by astronomers, biochemists, physicists and even social scientists.

In the summer of 1970 a conference took place at NASA's Ames Research Center to bring together acknowledged experts on this nascent subject. The presentations at that conference have now been made available in this book. Enthusiasts will recognise the names of the contributors—Sagan, Drake, Bracewell, Arbib, McCarthy, Aranoff, Oliver and Morrison, as well as those of the editors, Ponnamperuma and Cameron. The book is a well-balanced and eminently readable account of all the varying aspects of this intriguing and daring topic. Although this work constitutes a serious and in-depth study, including technical material of interest to experimentalists, the informal style and interdisciplinary profile of the subject matter will command a wide readership from non-specialists of many disciplines. (It also contains a 39 page bibliography.)

The feasibility of radio communication with extraterrestrial civilisations depends heavily on the proportion of stars in the Galaxy which are likely to support life-bearing planets with technical communities capable of transmitting, receiving and interpreting radio signals. Surprisingly, the most uncertain parameter which determines this proportion turns out to be a social one— the mean lifetime of technical communities. The contributors apparently share a strong opinion that the physical and biological conditions necessary for the formation of life are extremely common throughout the Universe and that given the appropriate type of star, intelligent life of some sort is almost inevitable. Carl Sagan comes up with a formula which predicts that the number of technical communities in the Galaxy is 10% of their mean lifetime in years. This implies the curious result that either the Galaxy is teeming with intelligent life, or, if our civilisation is typical, we are already doomed.

With the optimistic assumption that the technical phase lasts billions of years (there is plenty of time available since the Galaxy was formed) then there may be a few hundred million communicative planets around us.

The formidable problem which then emerges is where to look. Even the optimistic estimates indicate the necessity for a search pattern covering thousands of stars out to a distance of perhaps 1,000 light years—a daunting prospect. Nevertheless, in the early 1960s Frank Drake monitored two nearby stars at 21 cm wavelength in the famous Project Ozma, in the hope of detecting intelligently directed signals. A vastly more ambitious programme is

proposed by Bernard Oliver, arising from a careful study which culminated in Project Cyclops, a summer study at Ames in 1971 This project involves the proposal for the construction of a huge array of 100-m radio dishes spread out over about 100 km², to search a million stars out about 1,000 light years away This search might take many years to complete, but in view of the cosmic importance of the project, such a long term allocation of resources is deemed to be justified Oliver devotes part of his paper to the discussion of concrete proposals for hardware and experimental procedures The system could also be used for transmitting, but in view of the fact that we must be by far the youngest technical community in the Galaxy, it is assumed that the responsibility for this rests on other (extra-terrestrial) shoulders

Some lively discussion is presented about how two communities who know that they want to contact each other but don't know where to look ought to go about attracting attention Simple radio beacons are an obvious if rather unsubtle procedure Dumping a rare

metal into the local star to bewilder distant spectroscopists is another suggestion Bracewell discusses an intriguing conjecture that probes may be sent around the Galaxy to lie in wait near a hopeful system If radio signals are received it immediately beams them back as a delayed echo, thereby attracting the attention of an assured audience Novelties of this sort, though purely speculative, are nevertheless of great general interest and this book is to be thoroughly recommended as a good all-round introduction to the subject

One final point Most of the contributors reject the possibility of direct spaceflight between galactic communities because of the vast distances and uncertain destinations involved It is difficult to fault their reasoning Anybody who is uneasy about signalling other, possibly bellicose, intelligent beings need fear no invasion In any case, even Marconi's pioneering signals are now a mere few dozen light years out in space and very probably haven't yet reached any interested ears

P. C W. Davies

Action at a Distance in Physics and Cosmology By F Hoyle and J V Narlikar Pp x+266 (Freeman San Francisco and Reading, October 1974) £7 80

THE main purpose of this book is to demonstrate that physical facts which are usually explained using field theory can also be explained on the basis of action at a distance between particles

The book is concerned mainly with electrodynamics (both classical and quantum) and with the masses of particles, which are treated as arising from interparticle action in accordance with Mach's principle No attempt is made to give gravitation an action at a distance form and the identification of the gravitational field with the metric is retained

The direct interaction between particles is symmetrical in time, but to this must be added the effects of the 'response' of the rest of the Universe To be consistent one must obtain a cosmological model in which this response can lead to the usual purely retarded interaction In the case of the electromagnetic interaction this condition requires that the Universe be a perfect absorber in the future Of the usual models those satisfying this condition are static universes, the closed Friedmann model, and the steady state model' The static models can certainly be rejected on observational grounds, and most people, including apparently the authors of this book, would now also reject the steady state model In spite of the statement to the contrary on page 174 (which is based on the misleading principle of taking the Einstein–de-Sitter model as typical of the Friedmann models) the closed Friedmann model also satisfies the response condition for the mass field The authors, however, reject this model on the grounds that although retarded potentials are consistent, so are advanced potentials, so that the cosmological model does not define the direction of time Their conclusion is thus that the Friedmann models must be rejected altogether in favour of some more complex model, not specified in detail If, however, the choice were between abandoning the Friedmann models and deriving the direction of time from some source other than cosmology (such as thermodynamics) then I think most physicists would choose the latter

I have concentrated on the book's discussion of cosmology, since this seems to be crucial to the whole theory The book is, however, by no means written solely for cosmologists and it contains much to interest a wider audience I doubt, thought, that it will convert many to the action-at-a-distance viewpoint   **G. J Suggett**

Everyman's Astronomy Edited by R H Stoy Pp 493+48 plates (Dent London, January 1975) £4 50

ANYONE who has followed the development of astronomy over the past few exciting years will realise that this otherwise excellent book is about four years out of date Black holes are not even mentioned in the index, X-ray sources receive scant attention, and even the discussion of pulsars is most notable for its omissions Perhaps most seriously of all, given the readership for which the book is clearly intended, the recent probes to Mars (Mariner 9), Venus and Mercury (Mariner 10) and Jupiter (Pioneer 10 and 11) all produced their dramatic new data after *Everyman's Astronomy* was ready for the press

There is, of course, a reason for this unfortunate gap The volume, intended to replace the successful *Astronomy for Everyman*, was conceived in 1960 when several chapters were commissioned, but was later abandoned until 1969 when Dr R H Stoy was called upon to complete the project As he says in a preface, "the epoch of the book as a whole is approximately 1970", but further delay arose because of the need for extensive editing to ensure homogeneity and to reduce any overlap in contributions gathered over the best part of a decade

With hindsight, there is no doubt that the publishers would have done better to wait for another few years

and produce the definitive book of its kind for the late 1970s The present round of exploration of the Solar System is now complete, and there is likely to be a lull until Mars landers, and the passage of Pioneer 11 past Saturn, revive interest in the late 1970s In other areas of astronomy, the entire electromagnetic spectrum is now open to view from satellites, including γ rays, X rays and infrared radiation, so that again we are standing at a watershed in the development of the science

But the failure of the book to have caught just the right tide in the affairs of astronomy only assumes importance because what is covered here is covered so well With nine first rate contributors, including Professor V C Reddish, Professor Z Kopal and Professor David Evans, it would have been surprising had the text not been of a high standard The illustrations—photographs and line drawings—complement the text well, and with nearly 500 pages of lightweight paper packed into the volume of a pocketbook the only notable omissions are those caused by the publication delays

Not least among the assets of the book is its price, which puts it well within the reach of all serious amateur astronomers They would be well advised to acquire this book But even though it is aimed at such readers, not a few professionals (and certainly all astronomy students) are likely to find it valuable for its compact presentation of a wealth of basic data

John Gribbin

Semiconductors

Amorphous and Liquid Semiconductors Edited by J Tauc Pp ix+441 (Plenum London and New York, 1974) $33 60

INTEREST in the field of amorphous semiconductors has increased rapidly during the last 10 years because of the obvious commercial success of the Xerox process and the possible commercial success of the amorphous switching device Before that, technical interest in the amorphous state was sustained by those interested in photoconductors as potential light sensors and in glass technology, and fundamental studies derived largely from interest in the liquid state and in the development of general methods for defining order and disorder in solids

The graph of interest against time has probably now flattened off and major discoveries are appearing more slowly, meanwhile, several reviews of the field have appeared in book form It is a good time to ask if these books tell us whether we have got anywhere in understanding amorphous semiconductors, and whether it is worth sustaining any interest The questions come to mind because the first clear message from all of the works is that the fundamental problems of under-

standing amorphous solids are very difficult, and the experimental problems, such as control in preparation and the significance of measurements, are considerable Will the strenuous efforts which are still required to solve the problems repay us to a similar extent as, say, an equal amount of work on new crystalline semiconductors?

The collection of chapters edited by Dr Tauc certainly has the depth to give the reader a chance to address the question the extensive and thoughtful reviews are presented by some of the most respected workers in the field To this depth the editor has added a suitable breadth by selecting authors who have a suitable diversity of viewpoint Some freshness is added by the inclusion of authors who have not yet received very wide exposure

Bagley, a less well known author, draws a distinction between glasses and other amorphous materials, emphasising that the structure obtained from the cooling of a liquid will be "structurally and thermodynamically related to that liquid" but that similarities between those glasses and "other amorphous solids" may be tenuous Such a caveat is a useful corrective to carry into the other chapters, which seek mainly to explain the electrical and optical properties of that class of 'other amorphous solids'

Of the chapters which review large volumes of data, Tauc's and Fritzsche's are excellently condensed, although Tauc sometimes relapses into single-sentence listing without further observation and, in the end, shirks a conclusion Fritzsche, by contrast, ends his chapter on electronic properties with a precise and frank summary of the gulf which still exists between experiment and theory in the field of electrical transport He stresses that many amorphous semiconductor films contain heterogeneous voids and that this may invalidate many of the theories based on measurements of conductivity, elastoresistance and thermopower

In his second chapter, Fritzsche gives a summary of the chemical requirements for the components of a switching or memory alloy In such paragraphs, one feels that one is getting the distillation of a huge amount of research in a few sentences and that the book is thereby achieving its intention—that of a mature review

Girgorivici has managed similarly to condense knowledge in a lively review of the mode in which atoms pack and bond in known amorphous materials Econonomou, Cohen and others give a highly condensed summary of the necessary band and percolation theory

The book represents a useful reference volume and is a very good starting point for those who wish to understand the field   **A. G. Holmes-Siedle**

Affinity chromatography

Affinity Chromatography By C R Lowe and P D G Dean Pp xi+272 (Wiley-Interscience London and New York, September 1974) £6 25

THIS book would be a valuable asset to any research group working in fields such as enzymology and protein chemistry in which an awareness of the principles and potentialities of affinity chromatography must now be considered obligatory The original literature on affinity chromatography is now considerable and an introductory text was badly needed Both the length and the price of this book have been well judged

The reader should not be put off by the first part of the introduction in Chapter I as I almost was It consists of three pages on protein structure, which are entirely superfluous for anyone who is at all familiar with proteins, and which certainly would not help anyone who was not The flavour of those three pages is unfortunate, for example, the common acidic and basic groups of amino acids are wrongly assigned as in the worst undergraduate texts, peptide bond formation is stated to "occur" by elimination of a water molecule, of all things, and that is illustrated by a "lasso" in Fig I 1 The structure of the indole ring of tryptophan (Table I 1) is wrong and the hydrogen atom of the imidazole N–H bond is missing, as indeed it is throughout the book

Happily, after that the presentation improves dramatically Most sections provide a short but helpful discussion of theoretical aspects followed by interesting examples well illustrated by elution diagrams and by structural formulae where appropriate This is particularly helpful in a book that deals with many different types of molecules and which is aimed at a wide readership that must include people not normally used to thinking in molecular terms Although many of the theoretical sections are, of necessity, brief they do provide very useful starting points for further reading All sections of the book are well supported by literature references up to mid 1973 Following an introductory chapter, there are four main chapters These deal with the principles of affinity chromatography, group specific absorbents, various applications of affinity chromatography, including hydrophobic chromatography, and the organic chemistry of materials used in affinity chromatography, particularly the reactions involved in the preparation of various affinity adsorbents   **K. Brocklehurst**

To follow
a flow

Flow Visualisation By Wolfgang Merzkirch Pp viii+250 (Academic New York and London, October 1974) $26 00, £12 50

THE need for reliable and accurate methods for rendering fluid flows visible arises in a wide variety of situations in aerodynamics, chemical engineering and fundamental experimental studies in fluid dynamics Many different techniques have been developed over the years and major advances in the subject have occurred quite recently For that reason alone there has been a need for a book which collects together all these techniques and brings the subject up to date This book fills the need adequately In an exhaustive survey the author describes, with the appropriate theoretical background, the methods from many fields, and he identifies the conditions and parameter ranges under which each method is applicable

Merzkirch divides the subject into three classes over half of the book is given over to the classification which is concerned with the various optical arrangements available for obtaining both qualitative and quantitative information from fluid flows This classification, which is headed rather loosely "Optical methods for compressible flows" (even though, in fact, several of these methods have been successfully used in incompressible flow situations—schlieren systems in salt stratified fluids, for example) describes in detail techniques such as shadowgraph and schlieren arrangements which are based on the change in refractive index of a fluid as a function of density There are particularly interesting contributions on holographic flow visualisation and the laser-Doppler anemometer, though it is doubtful whether its inclusion in this book is strictly justified

The second division comprises techniques which rely on the addition to the flow of foreign material such as dye Attention is given to the basic criterion that the flow should not be disturbed by either the mode of injection or by the material itself, and a series of empirical rules, ranges of validity and necessary corrections are given for the various methods This section, which is descriptive in character, contains useful information on the more recent innovations such as temperature-sensitive paints, liquid crystals and flash photolysis, as well as coverage of the more traditional methods

The remainder of the book is taken up with a discussion of special problems and techniques and a third classification

headed "Flow field marking by heat and energy addition" The methods described in the book cover a very wide range but are confined essentially to laboratory scale flows For completeness it could have included some of the special techniques used in larger scale field observations (estuary flows or oceanographic phenomena, for example)

Nevertheless, the book is well written and contains a large number of excellent photographic illustrations, and it will be very useful to experimentalists working in many branches of fluid dynamics   **P. A. Davies**

Life time
of an organism

Living Clocks in the Animal World (A Monograph in American Lectures in Environmental Studies) By Miriam F Bennett Pp xiii+221 (Charles C Thoms Springfield, June 1974) $11 75

CIRCADIAN and other biological rhythms are attracting increasing attention every year Strictly speaking, however, we still do not know how their extraordinary 'clock' properties are achieved The majority of investigators, myself included, consider that the timing of circadian rhythms is derived from the chemistry and physics of the cell or organism At least one laboratory, however, led by Professor F A Brown, believes that the timing is provided by "subtle geophysical factors" associated with the solar day, and, incidentally, not excluded in most experiments

The author of this small volume has been one of Brown's associates, and when I opened the book I expected to find that she pressed the second of these views She presents both cases quite fairly, however, and interprets many of the phenomena in endogenous terms She points out that the 'exogenous school' does recognise that organisms are "equipped with cellular clocks", and that the 'endogenous school' recognises that environmental variables, particularly changes in light intensity and temperature, can modulate overt rhythms in a manner which is distinct from their rôles as entraining agents She considers, perhaps correctly, that a "spectrum" of clocks from "purely" endogenous to "purely" exogenous exists in the animal world

Her material is, however, limited to those groups which she knows best, and she ignores almost totally the work done on birds, mammals and insects, which has provided most of our knowledge on circadian systems For this reason, I would not recommend it to any of my students   **D. S. Saunders**

Biochemistry

The Development of Biochemical Concepts from Ancient to Modern Times
By Henry M Leicester Pp 286
(Harvard University Press Cambridge, Massachusetts, 1974) $15 00

THIS is a book which was very much needed, and happily Professor Leicester has made a real success of it Apart from a group of lectures at the Cambridge Biochemical Institute, recently published, the only thorough treatment of the history of biochemistry was the book by F Lieben, *Geschichte der physiologische Chemie,* published 40 years ago . Lieben's book has been reprinted in recent times and will always have its value, but it is not satisfying nowadays for a number of reasons For example, he disposed of the entire period down to the end of the 17th century in only 20 pages out of a total of 700, then he divided the main body of his text into physiological topics, which were followed by an account of discoveries in the present century grouped under proteins, carbohydrates and fats The attitudes of the early thirties are very dominant, and as a whole the work lacks historical perspective

This state of affairs is now vastly improved upon in Leicester's book Eighty pages are devoted to the period from antiquity to the end of the Middle Ages, 45 to the 17th century and the time of iatrochemistry, and the remaining 105 to biochemistry, as the child of modern chemistry and modern biology The book is well informed in classical and mediaeval history, yet comes down as far as the discoveries about the genetic code made by Watson and Crick in 1953, the latest references being to work in 1971

The discussion of the ancient Western world draws from the pre-Socratic philosophers and the Aristotelians, with a paragraph or two on Alexandrian 'alchemy' Far beyond the ken of Lieben, there is a good chapter on biochemical ideas in ancient China and India, now rightly regarded as necessary because of their great influence on Arabic culture, without a consideration of which the history of any science in post-mediaeval Europe can never properly be understood Leicester has a clear appreciation of their macrobiotic drive, as opposed to the more metallurgical aurifictive and aurifactive approach of the Hellenistic papyri and the Corpus writers, respectively The Arabic chapter is also well done, though perhaps the ideas in the Jābirian Corpus (p 56) are passed over a little too quickly On the other hand, it is a good idea to emphasise the dawning of the ideal of the necessity of quantification, which occurred in the late Middle Ages with men such as Nicholas of Cusa

It has been said that most of the early history of biochemistry can be followed by tracing all the peregrinations of certain fundamental ideas—such as *pneuma,* element, humour, *krasis,* quintessence, elixir, conjunction and ferment Only four of these are actually in Leicester s index, but two more of them are fairly readily found within the text, and the reader can easily add the references to the index in his own copy Although the Paracelsian chapter is so good, with its explanations of 'chaos', 'tartar', 'iliaster', and 'arcanum', one would like to have seen the concept of 'elixir' followed through somewhere, and again the ancient idea of the *conjunctio oppositorum* lying at the basis of all affinity theory, could also have been brought in with advantage The great influence of the first distillers of strong alcohol is relevant here, since *aqua ardens* seemed indeed to be an instance of the "marriage of fire and water"

As an offering towards a second edition, I would express regret at the attribution of the discovery of gluten only to Grimaldi in 1665 (p 118), since *mien chin* had been systematically prepared on a nation-wide, if not indeed an industrial, scale in China for at least a millennium before Indeed, the discovery may well go back to the second century BC if the traditional ascription to Liu An and his circle turns out to be justified I would also like to see a rewording of the statement on p 49 that a characteristic feature of Chinese medicine was its reliance on herbal medicines This is particularly misleading because the Chinese in their pharmaceutical natural histories from the second century BC onwards never shared the Galenic abhorrence of non-herbal remedies From the beginning their physicians made much use of mineral drugs, and animal products were 'officinal' from the beginning Thus, no Society of Chymical Physitians was ever necessary to awaken the Chinese from their Galenic slumbers, they had always been awake to the three kingdoms

A great attack on Galenic orthodoxy took place when the specific virtues and specificity of drugs, as opposed to the unspecific adjustment of 'peccant humours', were recognised in the 16th and 17th centuries (pp 86, 98) It might have been good to instance at this point the remarkable career of the Cambridge 'quack' physician, Talbot, who administered quinine widely and with great success in defiance of the reigning doctrine Still later we get involved in the vitalism-mechanism polemics of the 19th and 20th centuries Perhaps it is not quite enough to end the discussion (p 159) by saying that "with the accumulating mass of chemical and physiological information

vitalism gradually disappeared from biological thought" Would not a page or two of the current debates, centering round 'reductionism', have been in place here? They contain much of great interest for the philosophy of science, and the general position of organicism is so significant in the light of more than 2,000 years of past history, that this might well have been expanded Lastly, I should like to offer to the author for a footnote in his second edition, the tradition that the invention of the term 'hormone' (p 228) actually occurred in the Hall of Caius College at Cambridge, when Bayliss and Starling were dining at High Table and had a conversation about a word they were looking for with the Greek and Latin scholars who were of the company

Professor Leicester's book is well provided with exact references, the absence of which was one of the worst features of Lieben's book The new book can be recommended without reservations to students and research workers alike, but of course it is not the last word on the subject One can see a place which could be filled by something at one and the same time more philosophical and more industrially-oriented than either Lieben or Leicester, meanwhile let us be grateful for this book   **Joseph Needham**

Developing form

On Development: The Biology of Form.
By J. T. Bonner. Pp. 282. (Harvard
University Press, Cambridge, 1974.)
$10.00.

Do not expect to find here a fashionable account of developmental biology
in terms of molecular mechanisms controlling transcription and translation,
or specificity of cell surfaces, or automata theory. For this book attempts to
deal with developmental biology in a
very broad manner, by considering not
only mechanisms in a general way but
by considering also the problems of
evolution and population biology.

Bonner attempts to do this by
emphasising the importance of life
cycles, which he believes throws new
light on basic problems. He views, for
example, biological reproduction in
terms of reproduction of the life cycle
and this theme is expanded in the first
part of the book to discuss size increase in evolution, senescence, variation, the origin of life multicellularity.
An essential feature of a life cycle is
not only to reproduce a new cycle, but
to get rid of the old one, and he thus
argues for programmed senescence. (No
mention is made, however, of Hayflick's
studies on the limited life span of
somatic cells.) Some attention is given
to looking at life cycles as having points
of maximum and minimum size, and
size increase during evolution is discussed in some detail from the point of
view of selective advantage. There is
also a quite long digression on the
origin of social organisms. His aim is
to say something about the larger
significance of development, such as
why development occurs, how it
evolved, and its relationship to all other
aspects of life. Interesting though this
biology may be, I have not found viewing it in terms of life cycles very
helpful, nor do I consider it to be an
advance over the distinction between
genotype and phenotype. By looking at
the selective advantage of the phenotype he misses the really interesting
questions on the relationship between
evolution and development. What we
want to know is what constraints and
integrations development provides when
the genotype changes. How, for
example, do new cell types arise? What
is the selective advantage as they are
evolving? One can ask the same question of the evolution of structural
innovations such as the wing. Again,
what sort of changes in genotype are required in the alteration in the pattern of
muscular activity. Must one alter genes
controlling both nerve and muscle, or
will developmental mechanisms provide
the required integration? Could one by
appropriate selection evolve a bat-like
wing for man? Dobzhansky has said
no, and thus man has no hope of

becoming an angel, but I do not believe
current theory permits a decision. None
of these problems is even recognised in
this book.

The second part of the book is concerned with the mechanisms of development and covers topics such as
synthesis of substances, timing, localisation of substances and control of
pattern, and is rather disappointing.
Part of the difficulty is that Bonner
seems much more at home with
protozoa, fungi, slime moulds and
plants. This may account for the fact
that most of the examples are drawn
from such fields and not only are they
less familiar, but in general, much less
is known about them. This also
accounts for the rather cursory treatment of topics such as microtubules
and microfilaments and cell movement
in general. One could argue that for
animal development this was an area of
major importance in which rapid
advances are being made. Other areas
such as clonal analysis, which are being
applied with remarkable success to
insect development are not even mentioned.

The book is characterised in places
by a regrettable lack of rigour. For
example, the brief analogy drawn
between the brain and the structure of
the genome is very superficial, in that it
would be as true of a juke box. Again,
a principle of convergence is invoked
to suggest that particular biological
functions in development can be fulfilled in a variety of ways; but no
attention is given to a principle of
conservation which would suggest that
once embryos have found a good way
of doing things they will continue to
use it. In fact, one of the major failures
of the book is to bring out common
features and general principles in
development. Yet again, the author
suggests that it is a distinct selective
advantage for the grouping of developmental events into superunits of gene
action, but very little evidence is available to substantiate such a view: the
interconnectedness of genes remains a
major problem and is not seriously
analysed here. Later, he suggests that
there are not enough genes to account
for all individual neurones, completely
ignoring the tremendous combinatorial
possibilities of gene networks which
could easily and uniquely characterise
each neurone with a relatively small
number of genes.

In spite of these criticisms, the author
draws on a wide range of biological
examples and his ideas are often provocative and original. By raising problems ranging from the development of
the social behaviour of ants to the form
of mushrooms he presents to developmental biologists problems and ideas
that rarely cross their minds.

Lewis Wolpert

Seals, monkeys, apes and rabbit

A COMPREHENSIVE work on British seals is long overdue and one by an authority as eminent as the late Emeritus Professor H. R. Hewer is particularly welcome*. Although seals are among the largest of British mammals, before the last war comparatively little was known about them. Since 1947, however, an increasing amount of research has been undertaken and the published results form the backbone of this book. Hewer started his own research in 1951 and during the following years he visited all the major British colonies, with the result that this is no 'scissors and paste pot' work; it is something that not only bears the stamp of genuine knowledge, but also includes many personal observations.

The first chapter is devoted to a brief account of the Pinnipedia and their evolution into three dissimilar families; it also describes their anatomical features and shows how these have become adapted to a marine environment. The main part of the book concentrates on the life history of the two seals that breed in Britain, namely the grey seal *Halichoerus grypus* and the common (or harbor) seal *Phoca vitulina*, and it is a measure of the present state of knowledge of these animals that, whereas the grey seal warrants nine chapters, information about the common seal can be contained in two.

The remaining seals on the British list are also discussed and short accounts are given of the walrus and the four species of phocids that appear

as vagrants. Mention is made, too, of the occasional 'escaped' Californian sea lion.

A final chapter which deals with the vexed question of seal conservation and management, should be compulsory reading for all those who think of seals only as appealing white-coated babies. Five appendices and a comprehensive bibliography complete the book.

There are numerous illustrations and maps, some of the most useful being Hewer's own line drawings. The photographs vary in quality and, although some of the 'chalk and soot' can be excused on the ground of enlargement from colour film, at least two have already been reproduced much more successfully elsewhere.

My most serious criticism is levelled at Appendix D and, as the source of the basic data, I think it unfortunate that Hewer did not consider other possibilities before publishing his theories. There are other minor errors which someone with an intimate knowledge of a particular colony is bound to discover, but, these apart, *British Seals* is a book that can be recommended to anyone, whether amateur or professional, who is interested in these controversial mammals.

Grace Hickling

ONE of our most pressing problems, so we are continually told, is our aggressiveness. Let it rip and you may end up with a lifer, bottle it in and you get ulcers. The answer, they say, may lie with the monkeys. Although competition for food or attractive females is common in most natural groups and the majority of species possess the

equipment for easy murder, killing and serious wounding are rare. Holloway's book† provides no panaceas and includes little material of direct relevance to human aggression. It does, however, draw together current knowledge of several aspects of aggression in primates.

Seven chapters describe the patterns, distributions and contexts of aggressive interactions in different primate taxa: tree shrews, nocturnal prosimians, diurnal prosimians, ceboids, colobines, cercopithecoids and pongids. A further two describe the reactions of rhesus monkey groups to the introduction of additional animals. Two important, if obvious, themes recur throughout these chapters. First, aggression is the means by which individuals maintain access to food, females and territories. Second, the frequency, context and distribution of aggresive interactions differ between species, between populations of the same species and (though it is hardly emphasised here) between individuals; in most natural populations of primates, aggression is both flexible and adaptive. Both points need to be borne in mind when we consider our own predicament.

A general weakness of this group of chapters is that too much space is devoted to the description of trivial interspecific differences in aggressive behaviour and too little to the generalisations and theoretical implications arising from the surveys. The excellent chapter by Nagel and Kummer on cercopithecoids (Old World monkeys) is a marked exception and emphasises how much more is known about the behaviour of this group compared with the others.

Research on the effects of androgens, brain stimulation and neural lesions on aggressive behaviour is reviewed, and interspecific differences are related to variation in neuroanatomy. These reviews show that behavioural aspects of research in this field lag some way behind naturalistic studies in sophistication (though the automatic recording systems described by Maurus offer the possibility of a speedy reversal of the situation). The effects of physiological manipulations on aggressive responses given in different contexts or to different classes of individuals are rarely distinguished, and the numbers of animals tested are usually extremely small. This is an important weakness as there is evidence that the physiological controls of aggression are both complex and variable: that is demonstrated by the useful review of the effects of androgens by Rose *et al.*

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*British Seals. By H. R. Hewer. Pp. 256+24 plates. (Collins: London, November 1974.) £3.50.

†*Primate Aggression, Territoriality and Xenophobia*. Edited by Ralph L. Holloway. Pp. xiv+513. (Academic: New York and London, 1974.) $29.50.

Two final chapters by J. P. Scott and C. R. Carpenter review the field on a wider basis. Both are more concerned with easy generalisations about primate aggression—males are more aggressive than females, aggression occurs in competitive situations and is influenced by crowding or changes in group membership—than with important problems. That is a pity, because several theoretical problems occur throughout the book and require detailed consideration. Just what is aggressive behaviour? (It is defined in at least 10 different ways in as many chapters). Is predatory behaviour controlled by the same mechanisms or not? Should aggression include threats and displays or just physical attacks? What is the adaptive significance of differences in aggressiveness? If aggression is the means by which individuals maintain access to limited resources, why do they not kill subordinates and have done with it? When discussing the functions of aggressive behaviour, too many of the contributors treat aggression as a syndrome, suggesting advantages which invoke group selection. In contrast, there is too little discussion of the adaptive significance of differences in aggressiveness between individuals or between contexts.

Finally, how much do primate studies really tell us about humans? Clearly, research on the control and development of aggressive behaviour in primates suggests possibilities which can be investigated in man afterwards, but it is not obvious that broad, comparative studies help much. Perhaps the proper way to study mankind is not through monkeys after all since man's environment differs so widely from those of other primates. Despite these limitations, zoologists and comparative psychologists should be grateful to Holloway and his contributors for providing reviews which cover a wide and disparate literature.

T. H. Clutton-Brock

Primates: Comparative Anatomy and Taxonomy. Vol. 7: *Cynopithecinae.* By W. C. Osman Hill. Pp. xxxi+934+48 plates. (Edinburgh University Press: Edinburgh, April 1974.) £25.00.

THESE days, when half the new publications in science are either proceedings of symposia or multi-authored surveys of the latest scientific wonder, a scholarly monograph written by a single author is as welcome as the sound of the cuckoo in April. Volume 7 of Dr Osman Hill's encyclopaedic series on the primates is a worthy companion to the previous seven volumes (including Volume 8 which was published out of sequence).

It is not easy to criticise a work of such length and distinction without giving the impression of cavilling, but where matters of nomenclature and taxonomy are at stake even apparently trivial issues require comment. Two main criticisms spring to mind; there is a tendency to include everything rather than to select, and to cling to the past rather than to come up to date.

To illustrate the first point: two range maps of the same two species are included, showing radically different distributions (Maps 11 and 12, *Macaca arctoides* and *M. thibetana*). To say the least, that undermines the confidence of the reader.

The second point—clinging to the past—is instanced by the use of the name *Macaca irus* for the crab-eating macaque, ostensibly for the sake of uniformity with previous volumes. Since the publication of the *International Code of Zoological Nomenclature* in 1961, the zoological nomenclature game (once a highly esoteric pursuit) has become one which all can play. Anybody with access to a museum library can see for themselves that under Article 11g(ii) the name *irus* was not properly proposed by Cuvier in 1818, and will therefore reject it. The retention of an unavailable name merely tends to boost its status and prolong its use, thus perpetuating nomenclatorial confusion.

Turning from nomenclature to taxonomy, the same backward looking trend can be seen in Dr Osman Hill's handling of the *Macaca/Cynopithecus* controversy. In 1969 Fooden showed convincingly that the seven species (or subspecies) of Celebes macaques are monophyletic, probably an offshoot of Bornean *Macaca nemestrina* stock by way of the Makassar Strait. The species at the centre of dispersal, *M. tonkeana*, seem to be the least differentiated, and the terminal species in the north (*M. nigra*) and in the south-east (*M. brunnescens*) are, as might be expected, the most highly differentiated, and are linked to *M. tonkeana* by intermediate populations. Mayr, whose ideas on systematics and the origin of species have been widely adopted, would undoubtedly interpret these forms as congeneric. To quote from his book *Systematics and the Origin of Species from the Viewpoint of a Zoologist* (Dover: New York), "That they are nothing but subspecies, or at best allopatric species, is particularly evident in cases in which the widely diverging species are the extreme ends of a long chain of intermediate subspecies."

Nevertheless, Hill still feels it necessary to give taxonomic expression to the extreme differentiation of the northern Celebes 'Black Apes' (*Cynopithecus*) from the Moor Macaques (*Macaca maurus*), following Laurie and Hill (*List of land mammals of New Guinea, Celebes and adjacent islands, 1758–*

1952. Trustees of the British Museum, Natural History, London). But contrary to Laurie and Hill, the watershed between the two genera has been shifted further south so that *M. tonkeana* (considered by Laurie and Hill as a synonym of *Macaca maurus*) is now arbitrarily assigned to the genus *Cynopithecus*. Arbitrarily? I am afraid so, because no convincing data are given to back up this decision, without which it can carry no weight. It merely serves further to embroil the reader in the confused taxonomy of the group.

The present volume will undoubtedly be welcomed, particularly by applied scientists for whom the macaque monkey is still the centre of the experimental universe. The information and sources that Dr Osman Hill supplies are invaluable to all research workers who will continue to regard his series as the most authorative and definitive available. One will not see its like again.

John Napier

The Biology of the Laboratory Rabbit. Edited by Steven H. Weisbroth, Ronald E. Flatt and Alan L. Kraus. Pp. xi+496. (Academic: New York and London, 1974.) £23.75.

THIS is the first comprehensive text devoted solely to the laboratory rabbit, and it will prove to be of value to a wide variety of scientists concerned with the use of this species as an experimental animal, or who are engaged in the practice of laboratory animal medicine and husbandry.

There are 25 contributors (all from the USA) who provide 18 chapters. The text is supported by numerous illustrations and tables, together with a comprehensive list of references at the end of each chapter.

Chapters 1–3 consider basic aspects such as genetics, husbandry, anatomy, physiology and biochemistry. Additional genetic information is given in chapters 7 and 15, which are devoted to serological genetics and inherited diseases and variations.

More than half the book (11 chapters) is devoted to disease, parasitic infections and pathology, and successfully amalgamates much of the literature scattered throughout scientific proceedings and journals.

Chapter 4 is concerned with biomethodology and details many basic techniques and procedures.

Chapters 5 and 6 contain comprehensive reviews on the rabbit foetus in experimental teratology and on arterio-sclerosis research, and chapter 8 provides a sound introduction to gnotobiology.

Any attempt to consolidate information economically will leave gaps, and this book is no exception. Nevertheless, it can be thoroughly recommended for study and reference. **John Bleby**

Everything caudal to the eyes

Handbook of Sensory Physiology Vol. VII/3: Central Processing of Visual Information. Parts A and B. Editorial Board: H. Autrum, R. Jung, W. R. Loewenstein, D. M. Mackay, and H. L. Teuber. (Springer: Berlin and New York, 1973.) Part A: pp. xi+775; part B: pp. viii+738; DM 248 each.

NOT one book but seventeen (at the latest estimate), and each one so weighty that a single hand can hardly lift it. That is the *Handbook of Sensory Physiology*. It will probably exceed 13,000 pages with more than 4,000 illustrations and is undoubtedly intended to be a definitive statement of the progress of research on sensory systems. The list of authors is massively impressive, the production lavish, and the coverage catholic almost to the point of being indiscriminate.

Volume VII is devoted to vision and I am particularly concerned with Volume VII, Part 3, two large tomes which tackle the *Central Processing of Visual Information.* It is edited by Richard Jung, the father of the Freiburg school of neurophysiology, where work on the visual cortex began 25 years ago with the explicit motive of seeking explanations for perceptual phenomena in physiological terms. That theme is well represented in these books, which cover everything in the visual system caudal to the eyes.

Professor Jung opens Volume VII, Part 3, Part A, Section 1, Chapter 1 (believe it or not) with an encyclopaedic account of the successes of his own approach to the visual system, particularly concerning the role of so-called 'B' (brightness) and 'D' (darkness) neurones in determining the perception of brightness and contrast. This chapter alone is about 150 pages long and together with two other chapters of 100 pages or more (by Grüsser and Grüsser-Cornehls on movement perception, and van der Grind *et al.* on temporal transfer properties) it dominates the first book.

In Part B there are five especially valuable comprehensive chapters: Sprague *et al.* give a much needed, up-to-date account of the functions of visual projections to the tectum; Freund covers the physiology of the lateral geniculate nucleus; Brooks and Jung do the same for the visual cortex; and Szentágothai reviews the structure and ultrastructure of both the geniculate body and the cortex in two chapters that are as pleasing to the eye as they are to the rest of the nervous system caudal to the eyes.

It is the major reviews that give this work its historical importance, of course, but I enjoyed the books more for some of the shorter, more personal chapters on specialised topics: MacKay on the stability of the visual world, Levick on spontaneous and maintained neuronal activity, Gross on the inferotemporal cortex and Brindley on stimulation of the human visual cortex.

All these and many other fine chapters make the books indispensable for any library of physiology. Not that the whole venture is without fault. In general, the books suffer dreadfully from the lack of overall organisation that so often mars multi-author works. In fact, the degree of overlap and even straightforward contradiction between chapters, and the whimsically random systems of referencing make the books frustrating to read. The few chapters on visual processing in lower animals, although excellent in themselves, seem an incongruous sop to comparative physiology. Where is the essential review on the analysis of visual information (not just colour vision) in insects, spiders, octopus, crayfish and so on?

Finally, I am disturbed at the price: £45 for 800 pages. I believe that books of this sort ought not to cost so much; the libraries that will have to take the whole series are going to have to pay a vast sum. It raises in my mind doubts about the role of purely commercial enterprises in the essential business of disseminating scientific results. **Colin Blakemore**

Into the cell

The Cell Nucleus. Edited by Harris Busch. Vol. 1, pp. xxiii+667; vol. 2, pp. xxiii+564. (Academic: New York and London, July 1974.) $45.00; £21.60 each.

DR BUSCH has courageously attempted to collect together all the important knowledge about the eukaryotic nucleus and it has required 59 authors, many of whom have made fundamental contributions to their subject, to write the 39 articles. It is interesting to draw comparison with a similar major work, the six volumes on *The Cell* (Academic: London, 1959–64) which were almost exclusively devoted to the cytoplasm; and with a slim volume, *The Cell Nucleus* (Butterworth: London, 1960). Our knowledge then was quite primitive, but at about that time two fundamental observations were made which have led to the development of important new techniques and thus to many of the discoveries now described.

First, Marmur and others showed that it was possible to separate the two strands of the DNA molecule and then to reassociate them, a process depending on pairing between complementary sequences of the nucleotide bases. This

technique of reassociation, or hybridisation as it is called when the DNA chains are from different organisms, or involves an RNA chain, has led to a new understanding of the nature of the base sequences, their repetition and their location, in the very long DNA molecules thought to comprise each eukaryotic chromosome. In Busch's volume seven articles refer to various aspects of this problem and Strauss makes useful comparisons with the genomes of bacteria and viruses. The most thoroughly understood sequence is that coding for ribosomal RNA (Choi, Nazar and Busch), but the article on the fine structure of the site of its transcription, the nucleolus, may leave readers in a confused state because of the lack of a suitable schematic diagram and because of the variation in the terminology used for the different zones.

Second, in 1960, Barski discovered the spontaneous hybridisation of different cell types. Cell fusion has been called "the new gift to biology" and the reasons will become clear from Sidebottom's excellent article on heterokaryons. Cell fusion is the analogue of gamete fusion and Kucherlapti, Creagen and Ruddle describe its use in genetic analysis of somatic cells. The genetic content of the nucleus can also be studied by implantation into eggs and Gurdon summarises his elegant experiments which show that cell differentiation is not accompanied by loss of, or permanent alteration of, genes.

Five articles, including a lucid, basic introduction by Arrighi, provide a comprehensive survey of mammalian metaphase chromosomes and their recently discovered banding patterns. There are, however, numerous texts on these topics and the article showing clinical pictures of the effects of chromosome imbalance could have been omitted to help reduce the high cost of these volumes. Substantial articles deal with heterogeneous and other nuclear RNAs, nuclear proteins and DNA polymerases, and DNA-dependent RNA polymerases. Franke and Scheer, and Kasper, have contributed extensive and detailed articles on the nuclear envelope and their enthusiasm is evident from the number of recent results added in proof. There are also excellent chapters by Edström and by Hennig on polytene chromosomes. An aspect of chromosome structure, other than the base sequence, is the way in which the DNA molecule is folded up in association with proteins, and Solari skilfully steers us through the complexities, but unfortunately the book is already too out of date to include exciting new results on *v*-particles, nuclease-protected fragments and so on.

The Cell Nucleus contains most of the relevant material conveniently collected in one place (in addition to those mentioned the volumes include a number of other valuable articles) and will be an important source of references. Some subjects are, however, controversial—for example, the nature of heterochromatin—or complex—like repetitious DNA and its possible functions—and readers no doubt will feel a real need to consult the dozen or so annual reviews where related and sometimes more lucid articles can be found. Most chapters are very specialised and won't be suitable for undergraduate study.

There is no doubt that the books, which seem at first sight formidable in their bulk, would have benefited greatly from a good historical introduction outlining the important discoveries and showing how articles were chosen so as to fit into the general scheme.

Howard G. Davies

Plants and salts

Ion Transport and Cell Structure in Plants. By David Clarkson. Pp. xi+350. (European Plant Biology Series.) (McGraw-Hill: London and New York, April 1974.) £6.95.

This is the first book on plant–salt relations I have seen which takes full account of the advances in the subject which have been made in the last 10 years. In this respect it makes some recent American texts look distinctly old fashioned.

Here the undergraduate, for whom the book is intended, can read about isotope washout curves, the electrochemical approach and the chemiosmotic hypothesis, topics which have not been seen together before in a book of this type. The first six chapters are concerned with the cellular level and include an excellent chapter on the cell membrane. To illustrate the principles Dr Clarkson frequently refers to the alga *Hydrodictyon africanum* as a model; a good idea for an undergraduate text.

The second half of the book deals with the salt relations of the higher plant and in particular the root. It is not as well set out or as well written as the first half which one feels would make an excellent book on its own.

There are one or two errors in the text which might confuse the undergraduate such as the one on page 227 where in a discussion about the structure of the endodermis the reader is referred to a figure which turns out to be a scanning electron micrograph of a xylem vessel. Despite these blemishes Dr Clarkson is to be congratulated on his bold modern approach.   **D. J. F. Bowling**

Phycology and physiology

Algal Physiology and Biochemistry.
Edited by W. D. P. Stewart. (Botanical
Monographs, vol. 10.) Pp. xi+989.
(Blackwell Scientific: Oxford and
London, 1974.) £15.50.

MUCH basic physiological and bio-
chemical information on plants comes
from the study of algae, and the
general plant physiologist will be in-
terested in this book as a summary of
current knowledge on algal chemistry
and function. The phycologist's re-
quirement is also for a clear statement
of the algal situation, together with a
brief setting of the general scene so
that cross comparisons can be made
for major groups within the algae and
between algae and other plants. That
format is adopted by most of the
authors contributing to this book,
usually with marked success.

The book begins with a sensible com-
ment on biochemical taxonomy, appro-
priately enough by R. A. Lewin who
edited the 1962 *Physiology and Bio-
chemistry of Algae*, now superseded by
the present volume except as a survey
of the earlier literature; I am glad to
see Dr Lewin warn against the dangers
of generalising from studies on one
strain of one species under one set of
conditions, and of making comparisons
at class level from knowledge of a few
species out of thousands. This is fol-
lowed by comprehensive chapters on
cell walls and polysaccharides, storage
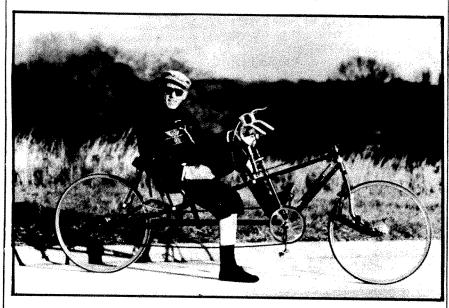
products, fatty acids and lipids, sterols
and nucleic acids. Each contains a
wealth of information correlated to the
biology and taxonomic position of the
algae concerned. In a chapter on
nuclear and cytoplasmic inheritance in
green algae, Ruth Sager confines her
account almost exclusively to investiga-
tions on *Chlamydomonas*. The result is
a chapter of substance by the expert
in the field and, since a forthcoming
title in the Botanical Monographs
series is a 250 page book on *The
Genetics of Algae*, Dr Sager is obvi-
ously right to present this account
rather than a telegraphic review of all
algal genetics.

Unfortunately, one or two chapters
attempt to summarise overlarge topics
and fail for lack of space. In the chap-
ter on cytoplasmic organelles, L. V.
Evans tries to review the relationship
between the structure and function of
f l a g e l l a, haptonemata, trichocysts,
scales, lysosomes, microbodies, con-
tractile vacuoles, walls, nuclei, micro-
tubules, nuclear division, mitochondria
and Golgi bodies, all in less than 20
pages of text. The task is manifestly im-
possible and the result is a catalogue
of truncated descriptions and oversim-
plified generalisations that seem out of
key with the rest of the book. Three
pages are devoted to descriptions of
mitotic ultrastructure in various **algae**
but there is no comment on signific-
ance, either functional or phylogenetic.
Golgi structure and function receive
better treatment but it is a pity that a
separate chapter could not have been
given to this topic, allowing room for

some interpretation and ideas in the
account of other organelles. Plastids
are given a whole chapter in which T.
Bisalputra is able to discuss, albeit
briefly, variations of chloroplast ultra-
structure in relation to algal classes and
pigments, and this is followed by chap-
ters on chlorophylls, carotenoids
and biliproteins, which satisfactorily
tidy up many of the earlier conflicting
and doubtful records (especially for
carotenoids).

Moving from biochemical to physio-
logical topics, we come to a series of
excellent chapters on light absorption,
emission and photosynthesis, photosyn-
thetic electron flow—with a fascinating
small follow-up chapter on the elucida-
tion of the electron transport chain by
the study of algal mutants—and a wide
range of other topics. Physiologists may
fight among themselves over some of
the more controversial ideas expressed
in these chapters but personally I found
them, not surprisingly, highly informa-
tive and, more surprisingly for a non-
physiologist, largely intelligible. In
cases where the technical aspects of
the subject are difficult or, frankly,
beyond me, I still found that most of
the authors were able to convey to me
the general significance of the studies
under discussion.

In a short review one can do little
more than indicate the main coverage
of the book and comment on its
general standard. The coverage is wide,
the general standard excellent. If there
are any significant errors of chemical
fact these will doubtless be commented
on in other reviews by biochemists.
There is a discrepancy between Evans
(pp. 96-7) and Nultsch (p. 866) in the
information on microtubule subunits,
even allowing for the fact that 4.5 μm
is a misprint for 4.5 nm in the latter
account; Prasinophyceae are charac-
terised by scaly flagella rather than
hairy flagella (p. 3); other errors and
misprints are few and far between.
Production is good, though some of the
electron micrographs are overenlarged,
badly printed and chopped off at the
edges. The price is high, but that is to
be expected for a 1974 book of nearly
1,000 pages.

As a nonphysiological, nonbio-
chemical phycologist, I found some
parts of this book easy and enjoyable
to read and other parts heavy going.
In relation to my research, teaching,
reading and editing interests in the
algae, the book has already proved in-
formative and stimulating. The data
summaries, formulae, tables and dis-
cussions will be invaluable for refer-
ence purposes. There is no doubt that
this book will be an essential work of
reference for years to come, both for
the phycologist and the p l a n t
physiologist.   **Gordon F. Leedale**

Captain Dan Henry, supine upon his recumbent bicycle. Captain Henry, from
Flushing, New York, became well known for his early design of sprung bicycle.
His adaptation, though somewhat cumbersome by today's standards, rendered his
machine "faster in hill climbing", and he was able to note with some satisfaction
that his tyres lasted longer. Energy conservationists take note. From *Bicycling Science,
Ergonomics and Mechanics*. By Frank Rowland Whitt and David Gordon Wilson.
Pp. xiii+247. (MIT Press: Cambridge, Massachusetts, and London, 1974.) n.p.

Biochimie. Edited by François Chapeville and Hubert Clauser. Pp. 872. (Hermann: Paris, 1974.) Fr. 178.00.

THE appearance of a comprehensive new textbook of biochemistry in French may well be met by English-speaking biochemists with an equanimity verging ever on indifference. For all that, it is an event of no little note, for creeping Americanisation has been threatening increasingly the character and individuality of European science. In most areas of research the European languages have been all but displaced by the new *lingua franca*. Very recently a discovery in a French laboratory was given wide publicity in the press, and *Le Monde* thought it worth remarking, in a tone of apparent asperity, that the work was being published in the American journal *Proceedings of the National Academy of Science of the U.S.A.* (rather, by implication, than in the corresponding French organ), and in French.

That this comment should ring so quaintly, shows how completely the realities of the situation, unpalatable as they may be, have been accepted by French (and no doubt other European) scientists in recent years. If English is the currency of research, however, it must nonetheless not be allowed to become the exclusive language of science. If French science is to preserve its own distinctive flavour and personality, matured through centuries of tradition and evolution, the language must be kept alive in the science faculties and textbooks. That in itself seems to me good reason to be grateful to the publishing house of Hermann, which has followed its admirable series of silver paperback monographs with a substantial new text, edited by Chapeville and Clauser and comprising separate contributions from 11 authors.

The publisher has secured a distinguished line-up of contributors, who have provided a well-balanced coverage. The book deals with bioenergetics, reaction mechanisms, proteins and amino acids, enzymes and catalysis, structure and metabolism of the saccharides, lipids and steroids, nucleotides and nucleic acids, intermediary metabolism, oxidation, phosphorylation, photosynthesis, biosynthesis of metabolites, and the chemistry of heredity. The treatment throughout is authoritative and clear. Some of the authors lean to a conservative view of what constitutes the established canon, and tend, therefore, to stress the classical rather than the topical.

The book is altogether not free from the inevitable drawbacks of multi-author treatises; in particular one

misses the unifying thread that a single author can at best impose by his view of the subject. There are also places in which the fields of interest do not interlock. For example, there is no mention of muscular contraction, chromatin, or nervous conduction, and the word rhodopsin is not in the index. But these lacunae are almost universal in the standard text-books in English, and I do not wish to cavil with what strikes me as a notable achievement. All of the authors have long teaching experience behind them, and many

French versions

generations of undergraduates and graduate students (myself included) have sat at the feet of most of them. They seem to me to have fulfilled their tasks admirably. And though it would be invidious to draw comparisons my personal choice for style and stimulus would be the chapters written by the two editors.

The book is attractively laid out in the modern style, with elegant typeface and attractive diagrams in pastel shades, which I hope will now generally supplant the rather stately and oppressive format, too often characteristic of scholarly works in European languages. I do not doubt that it will benefit French undergraduates, medical students and research workers for many years to come, and one may hope that translation may also render it ultimately accessible to English readers.

Anne d'Albis

Geologie de la France. Vols 1 and 2. By J. Debelmas. Pp. 1–294, vol. 1; 295–539, vol. 2. (Doin Editeurs: Paris, 1974.) Fr75.00 each.

THIS book is a series of articles written by various specialists, each of them working on one of the natural geological regions of France. In the preface, Jacques Debelmas, who dealt with the coordination of the various articles, states the ambitions and limitations of his undertaking: ". . . the problem is not only to give a perspective of the stratigraphy, and the tectonics of the various regions, . . . but to link both, so that it is possible to recognise a structural evolution through a succession of features and, on the other hand, to use this evolution when it is known, for a better understanding of these features through time and space . . .". There is another requirement, conciseness: ". . . it is a mainly didactic introduction to the geology of

France". This is not a treatise, but more a manual of regional geology for the use of students, which excludes numerous discussions and hypotheses, and long bibliographical lists.

The book is divided into two volumes. In the first, after a general introduction to the geology of France, the reader will find a description of the "Ancient massifs" — the Ardennes, Vosges, Armorican Massif and Massif Central—and a study of the vast sedimentary basins—the Paris Basin and the Aquitaine Basin—in which the pre-Mesozoic structure is depressed although the secondary and tertiary layers are only slightly deformed. In the second volume, there is a description of the folded ranges of the Alpine cycle: the Pyrenees, Provence, the Franco-Italian Alps, Jura, Corsica, Burgundy and the Rhone valley and Languedoc.
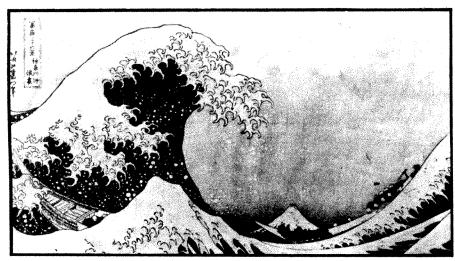
One is left with two impressions. First, the work has achieved part of its aims: because of its legibility, many illustrations, logical presentation, and also because of the care of the authors to reach the quintessence of their subjects, the book is both easy to read and interesting, and its success among students is assured. It is very convenient to have, assembled in one book, records of the large number of geological studies made in France since the last century.

The second impression which remained was of the disappointing lacunae within the book. All discussion of plate tectonics is set aside. Certainly the reader is made aware of this in the preface; but is it really defensible to omit a subject which has revolutionised earth sciences in the last few years? In addition, surface geology, easily observable, is highlighted at the expense of depth geology, which demands the indirect methods of geophysics; for example, no information is given on the thickness or structure of the crust. An analysis of seismology (which could have introduced a chapter on neotectonics), and of the interpretation of magnetic and gravimetric ideas, though essential to all structural studies, are missing. Finally, the presentation of the deep structural levels of the "Ancient massifs" does not pay enough attention to the recent results of microtectonics, the study of metamorphism (facies and facies series) and geochemistry of magmatic rocks.

This is an instructive and attractive book; but perhaps it will give students too incomplete a picture of the geology of France; and it may give foreign readers a false impression of French research in the earth sciences.

G. Boillot and R. Capdevila

Finding answers from the seas

The Sea: Ideas and Observations on Progress in The Study of The Sea. Vol. 5: *Marine Chemistry.* Edited by E. D. Goldberg. Pp. xiv+895. (Wiley-Interscience: New York and London, June 1974.) £21.20.

AT the First International Oceanographic Congress in New York in 1959, Lars Gunnar Sillén, Professor of Inorganic Chemistry at the Royal Institute of Technology in Stockholm, was invited to express the views of a laboratory chemist on the composition of sea water. His lecture, "The Physical Chemistry of Sea Water" represents a milestone in the development of ideas about the chemistry of sea water and the evolution of the composition of the hydrosphere, atmosphere and lithosphere over geological time. As Goldberg points out, the lecture was, in fact, the first of a series of investigations of the implications of equilibrium calculations of the ocean system, which challenged many prevailing notions and provided new ideas about a wide range of natural phenomena.

Volume 5 of *The Sea* is dedicated to Sillén and consists of accounts of the status of specific aspects of marine chemical research and includes chapters on completely new growth areas which have developed since Sillén's passing.

The status reports are largely contained in Part I (Thermodynamics of the Seawater System) and Part III (The Sedimentary Cycle). Millero, Disteche, Dyrrsen and Wedborg review the purely thermodynamic aspects of sea water as an electrolyte solution. Gieskes discusses the alkalinity/CO_2 problem, and confirms the confusion that arises over the use of apparent constants to describe the CO_2 system in the sea. That chapter is particularly refreshing because in addition to thermodynamic and kinetic considerations, mention is made of the influence of both sediments and purely physical oceanographical processes on the distribution of $CaCO_3$ and some other chemical parameters in the sea. Breck reiterates his own suggestion that the pE (the redox potential) in oxic seawater is governed by the O_2/H_2O_2 couple and has a value substantially different from that estimated by Sillén.

Part III is by far the largest section of the book, and starts off with a chapter by Garrels (who specifically mentions the effect of Sillén's 1959 lecture on the direction of his own research) and Perry. It is a brief summary of the main conclusions of several papers by Garrels and his co-workers, and of the book *Evolution of Sedimentary Rocks* by Garrels and Mackenzie (1971). The section also has two interesting and quite different chapters on specific problems in sedimentary geochemistry, namely magnesium (Drever) and silicon (Wollast). Chapters on diagenesis (Berner), interstitial waters (Manheim and Sayles) and sulphur (Goldhaber and Kaplan) emphasise the large amount of attention currently directed towards post-depositional reactions in unconsolidated sediments, reactions which are important in controlling the long-term composition of sea water itself.

In Part IV (The Impact of Life Processes Excluding Man), Menzel gives a timely, if partisan, review of the mass of conflicting data on the distribution of dissolved and particulate carbon in the sea, and its metabolic fate. A large amount of data obtained about 10 years ago, which was treated with some scepticism when it first appeared, is apparently reliable so that some early, hotly debated, suggestions about the carbon cycle are probably correct. The relatively new area of the chemistry of marine natural products is reviewed in depth by Faulkner and Andersen, and Lowenstam offers a lengthy account of the information available on the chemical composition of inorganic skeletal material and the impact of skeleton building on other chemical and physical processes in the oceans.

Part V (The Impact of Man on the Chemistry of the Oceans) is remarkably short in view of current interest (albeit concern) in the topic and the influence that the editor has himself had in drawing attention to it. The chapter by Jernelöv on heavy metals, metalloids and synthetic organics is very brief and inadequately referenced, and that by Preston dealing with artificial radioactivity is simply an inventory and a straightforward account of the possible behaviours of important radionuclides. Part VI (Origin of the Ocean) contains a single chapter by Arrhenius, De and Alfvén, which reviews their own recent work in several far flung branches of space research.

The most interesting contributions in this large volume are, however, contained in Part II (Air-Sea Interactions). They are interesting because they are concerned with new growth areas in marine chemistry. Thus, MacIntyre presents an iconoclastic, in places amusing, and thorough review of reactions in the sea–surface microlayer using a mass of data drawn from sources not immediately available, or indeed obvious, to marine scientists. Seiler and Schmidt, in a refreshing account of non-conservative gases in the sea, point out that the main source of nitrous oxide in the atmosphere is in fact the ocean, and that the atmospheric budgets for hydrogen, methane and carbon monoxide are affected to important degrees by processes in the sea.

This volume, although it is an invaluable addition to the series, does have some deficiencies. Specifically, the success or otherwise of the ion association and specific interaction models in describing the properties of sea water electrolytes is passed over rather swiftly. Recent work by Whitfield, Leyendekkers and Pytkowicz, for example, has given interesting new insight into the utility of some of the available models. A second almost completely neglected area which is only mentioned somewhat obliquely by Gieskes and Menzel, concerns the currently popular use of advection–diffusion models to describe and to understand the distribution of certain chemical parameters in the oceans. Whatever one may think of the usefulness of this particular approach, it has focused some much needed attention on some of the hidden effects that physical and biological processes have on the behaviour of some of the variables frequently measured by marine chemists.   **S. E. Calvert**

Deep Sea Sediments. Edited by Anton L. Inderbitzen. Pp. ix+497. (Plenum: New York and London, 1974.) $42.00.

THIS volume, resulting from the proceedings of a symposium conducted by the Ocean Science and Technology Division of the US Office of Naval Research in 1973, is remarkably comprehensive. The 23 papers achieve more than adequately the purpose of the book, to bring into sharper focus the "state-of-the-art" regarding the physical and mechanical properties of sediments and the need to establish some degree of coordination among investigators. What has had to be left out has been amply included in the references at the end of each paper which, in total, comprise a truly universal bibliography in the field of sediment mechanics.

From a panoramic viewpoint the volume deals mainly with the present situation regarding sampling devices, laboratory measurements (both mechanical and physical) and the extension of laboratory investigations to the field.

In the Atlantic, Indian and Pacific Oceans, physiographic provinces have been devised as a means of establishing some order to the quantity of mechanical data which is becoming available. Empirical equations relating mechanical and physical properties have shown minute spatial variations within each province, and carbonate distribution studies may explain some of these variations. It is, however, stressed that the provinces are not homogeneous entities and that although the mechanical/physical correlations may indicate trends, overall correlations are less accurate than those obtained for single provinces.

A number of papers deal with an extension of the correlations from laboratory/laboratory to laboratory/field, and serve to indicate that, at present, the prediction of *in situ* sediment properties and of the settlement of structures to be placed on the sea floor are woefully inadequate, with up to 30% error under ideal conditions of measurement.

Therefore, questions are posed concerning the measurements being taken. Is the macroscopic approach of the engineer good enough? Shall we ever achieve good all-round working prediction models using these macroscopic methods? The safety factor of the engineer conceals a lot of what is lacking in theory. Thus, it seems that more effort is required in a study of microstructure—so that the engineer's methods may become modified for the sediment researcher.

The urgency of the situation is stressed amply in these papers which indicate the imminence of commercial enterprise in the mining of ferromanganese deposits as a source of

Natural Gases in Marine Sediments. Edited by Isaac R. Kaplan. Pp. viii+324. (Plenum: New York and London, 1974). $30.00.

THIS volume consists of 17 chapters, which originally derive from papers presented at a symposium held at Lake Arrowhead in November 1972. The various possible sources of gas in sediment are discussed in the early chapters, together with such analytical information as is available. Because of sampling difficulties, most of the data refer to shallow water cores or to certain Deep Sea Drilling Project sites; by now, much of this information has been available in the literature for some time, but it is useful to have it presented in one volume, if only because it emphasises the restricted range of data available.

The latter half of the book is given over to consideration of the physical state of the gases in deep sea sediments, with particular regard to the possibility that gas–water clathrate compounds, or 'gas hydrates', may occur under the *in situ* conditions of low temperature and high pressure. Three chapters on the known thermodynamic properties of such compounds establish the areas of the world ocean in which these ice-like solids are stable; and as gas concentrations must be very high, some mechanisms, such as the *in situ* production of methane by the bacterial decomposition of organic material, or the injection of carbon dioxide, hydrogen sulphide, or methane from geothermal vents, must also be available.

Given that, the possible zone of hydrate occurrence is bounded on the upperside by the increase of temperature and decrease of hydrostatic pressure as the sea surface is approached from below and on its lower side by the increase in temperature

resulting from the geothermal gradient. For instance, in sea water which is 2 km deep, and which has a bottom temperature of 2 °C, it is shown that methane hydrate would be stable to a depth of about 600 m within the sediment. Although the hydrate would theoretically also be stable in the water column above the sediment at all depths below about 500 m, it is not likely that the required gas concentrations could be sustained outside the sedimentary column. Thus, the phenomenon is to be expected only within the upper regions of deep sea sediments.

Although no unequivocal proof of the existence of gas hydrates is given, three chapters describe the occurrence of sub-bottom acoustic returns which could be interpreted in such terms. Two authors also describe laboratory rigs for the formation and study of hydrate–sediment mixtures, and preliminary results are given.

One editorial function, which is particularly difficult with symposium volumes, is to ensure that a subject is covered evenly and without unnecessary repetition. The editor of this volume has included a useful and well written introduction which draws together the threads of research presented by the various contributors; it is a pity that he could not at the same time have eliminated redundancy. It is, for instance, irritating to be told five times of Humphry Davy's discovery of chlorine hydrate. The appearance of the book, apparently produced by a photo-offset process from a very superior, accurate and uniform typescript, is beyond reproach. *Natural Gases in Marine Sediments* can be recommended to all those interested in marine sedimentation, as a useful introduction to an under-explored field of study.

T. R. S. Wilson

copper, nickel and cobalt and which consider the resulting public concern over the protection of fauna and environment.

There is a need for better instruments for obtaining undisturbed samples. Box covers seem to give good samples but are at present limited to the near surface (36 cm). There is, however, an even greater need for undisturbed material from greater depths. The transport of core material, as well as the actual sampling procedure, produces varying degrees of disturbance. That, and the weakness of prediction models, has stimulated more *in situ* work.

Yet the contributors have not forgotten that their research is often

limited by economic stringency and that laboratory work relating to the deep sea environment must always play a vital role.

It is, however, pointed out that economic constraints can be lessened by means of a coordinated approach to sediment research.

There is a call also for the standardisation of techniques, symbols and units of measurement, concerning which this volume has put forward many worthwhile proposals. Standardisation would necessarily save much time and confusion.

The bibliography, data, problems and ideas presented in this volume will be invaluable for future work in sediment mechanics.   **Sinclair Buchan**

Living ancient history

Introducing Geology: The Earth's Crust Considered as History. By D. V. Ager. Pp. 256+21 plates. Second Edition. (Faber and Faber: London, February 1975.) £3.95, hard bound; £1.75, paperback.

THIS is a book about very ancient history. It reviews, in readable language, the geological evolution of the British Isles, with some references to other parts of the world. Since the first edition appeared in 1958, there has, as everyone knows, been a revolution in the earth sciences: continental drift has now been proved, and seafloor spreading, subduction and plate tectonics have all become recognised as aspects of the surface layers of a planet that is probably expanding. This new edition adds a bold but sufficient outline of these exciting developments to the first chapter and applies them at later stages; for example, the mid Palaeozoic troughs are seen as resulting from the opening of a proto-Atlantic between plates bearing America and Europe on their backs; plates which later closed to produce the Caledonian Mountains. Similarly, the break-up of the former southern continent of Gondwana under the influence of convection currents in the upper mantle is used to illustrate an important stage in geological history. In that and other ways, the reader is introduced to modern ideas of global tectonics.

The general treatment remains the same: the consideration of groups of geological epochs and systems. Thus, the chapters entitled "Oldest Rocks of All" covers the 4×10^9 years of Precambrian time; the "Oldest Rocks with Fossils", the Cambrian, Ordovician and Silurian systems; "Dry Land and Shallow Seas", the Devonian and Carboniferous. Each chapter is illustrated with maps showing the general outcrops of the relevant strata in Britain; and palaeogeographical diagrams, some original, some based on the works of L. J. Wills and F. W. Shotton, are included. There are illustrations of typical fossils but there is a minimum of fossil names and almost no mineral names. The chief rock-making and physiographical processes are described as they become relevant and they are illustrated with well produced plates, prepared mainly from Geological Survey photographs.

In one respect, Professor Ager has again been overtaken by events. His chapter entitled "The Long Quiet Episode", devoted to the Rhaetic, Jurassic and Cretaceous, was very suitably named when he prepared this second edition; as he says, it was reasonable to think of the European plate sailing peacefully at this time. But last November, the North Sea petroleum operators released their geological results, showing that the later stages of Jurassic time were not all quiet in the British area. There were in fact major rift-forming movements in the centre of what is now the North Sea, accompanied by modest volcanic activity and followed by extensive erosion. The rift profoundly affected Cretaceous sedimentation, which had filled up the trough by the time that the Tertiary basin covered it. The effects of these 'Kimmerian' movements explain the absence and reappearance of Jurassic strata in various parts of the North Sea Basin, and make us realise that the apparent continuity of the Jurassic outcrop across England is no indication of what happens in the third dimension. There will be material for a third edition of this book, but in the meantime it is none the worse for having missed the Kimmerian. Geology is, after all, a living and developing subject, and this historical approach to the country which so much influenced its progress is a good introduction to it.

Kingsley Dunham

By jove

The Jupiter Effect. By John Gribbin and Stephen Plagemann. Pp. 136. (Macmillan: London and Basingstoke, 1974.) £3.95.

" . . . all we have done is to gather in one volume data from scattered sources and look at them anew . . . "

And a splendid job the authors have made of weaving a complex, yet neat, design from the geophysical observations they have used. There may be a piece or two still missing, and the picture might well fall apart when lifted by the corner, but nonetheless it is a readable yarn which inquisitive citizens will enjoy; and seismologists may envy the authors' courage in undertaking to predict that the next great earthquake on the San Andreas Fault will occur in 1982, give or take a couple of years.

In that year the planets of the Solar System will be in conjunction with every other planet for the first time in 179 years. On the evidence of correlations—observed since the 17th Century—between peak sunspot activity and peak tides raised on the Sun, the authors predict a dramatic increase in activity for 1982. This, they argue might initiate a chain reaction through the interaction of solar wind and cosmic rays with the upper atmosphere, thereby affecting world wide atmospheric circulation patterns which in turn would change the Earth's rate of spin to provide the relatively small triggering force required to raise the 'normal' level of seismicity in highly strained belts. Observational evidence is well documented, and the circumstantial links in the chain plausibly argued. In the process the authors have provided lay readers with an educational glimpse of several geophysical processes and the geophysicist with sufficient stimuli to follow their suggestion to "wonder how such an interaction *could* work".

Why pick especially on the San Andreas Fault? Well for one thing it must attract wider interest (and a larger market) than, say, the Highland Boundary Fault and for another it is so well studied, so closely knitted instrumentally, that even the seismologists concerned are confident enough to venture predictions (though none so closely as 1982 ± 2 years). The latest of these professional forays into the fashionable field of earthquake prediction was published in *Nature* a few months ago and rules out the area between San Francisco and Parkfield for a really large earthquake before 1999, but accepts the possibility of a moderately large disturbance from 1981 onwards. Those predictions were based on recent observations of the rate of change of P-wave velocities, on which readers are brought up to date in an appendix to *The Jupiter Effect* (a title which neatly fits a book that unashamedly and provocatively attempts a geophysical explanation for the predictions of astrologers). Gribbin and Plagemann did not have time to fit this more recent work into their jigsaw and, in any case, they persuade us by other published evidence that the area on the San Andreas which is primed for triggering lies nearer to Los Angeles, south-east of Parkfield.

Even if such forecasts turn out to be less precise than hoped for, there is little doubt that California, as well as other thickly populated seismic areas, will suffer from great earthquakes which cannot be avoided unless lubrication can be applied to smooth the rate of release of stored energy. And as it would be a very brave (or ill judged) decision to pump water along the San Andreas Fault, the authors return to the unglamorous duty of spelling out the precautions which town planners and individuals can take to mitigate the inevitable effects. That their common sense statements of the obvious are considered worth printing demonstrates the social nature of the problem. The most sophisticated of observational and analytical efforts will contribute nothing to ease the disaster confronting a dam which, on the face of it, is intended to staple the edges of the Pacific and North American plates.

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as previously thought, a more or less accessory effect of extracellular secretion. Endolithic cyanophytes may even turn out to be 'rock-eaters' in the true sense.

Scanning electron microscope (SEM) studies of borings of a filamentous blue-green alga of the *Hormatonema* form[1-3] revealed the marks of a precisely controlled excavating process, suggesting the existence of specialised boring organelles in the alga (Fig. 1). Indeed, the discovery in the cyanophytes of very refined adaptations to an endolithic mode of life should hardly be a surprising find, since these organisms, according to palaeontological data, have been boring into calcareous substrates at least since the early Ordovician (that is, for about 500 Myr) and perhaps much longer than that[4,5].

The boring mechanism of endolithic algae has previously been described as an extracellular dissolution process: acid or chelating fluids, released by the terminal cell, supposedly dissolve small volumes of the mineral substrate and the algal filament penetrates step by step into a tunnel formed by a sequence of small hollows[3,6]. How the fluids are removed from the inner end of the inhabited tunnel, whether by extracellular circulation/diffusion or by metabolic uptake and intracellular transport, is not known. In the dissolution process, the micromorphology of the excavated space is determined by the solubility characteristics of the bored substrate and is thus beyond the immediate control of the organism. For example, the void space in bored calcite spar replicates the calcite rhomb[6], and borings in dolomitic limestone may contain residual crystals of poorly soluble dolomite which has resisted the attack[7].

The material described here derives from saline rock pools in the upper swash zone in modern beachrock west of Stazousa Point on the northern coast of Cyprus (see ref. 8). The bored rock consists in part of a coarse crystalline limestone, with optically uniform calcite crystals, several hundred micrometres across. These crystals provide excellent substrates for the study of algal borings by means of SEM.

The endolithic algae consist of dark blue filaments, about 300 μm long and 10 μm in diameter. Exposed to acid solutions the dark blue pigment turns reddish-brown, a reaction considered typical in *Hormatonema*[3,6]. In isolated colonies consisting of a few filaments, the algae grow radically away from a common starting point whereas in crowded colonies parallel filaments grow perpendicular to the rock surface. These arrangements are consistent with the observation that algal filaments commonly repel each other and avoid connecting with other tunnels[9].

The cavity formed by an individual filament is a fairly straight tunnel, replicating the organism. Branching of tunnels has not been observed.

As reconstructed from the microtopography of the tunnel walls, the boring process proceeds through the etching out of small grooves, about 1 μm in width and depth and 10 μm in length (Fig. 1). The wall relief increases slightly behind the terminal cell. The orientation of the grooves is approximately at right angles to the long axis of the tunnel. Interestingly enough, there are two laterally opposite sets of grooves, meeting in a zone of small pyramids. The arrangement indicates a bilateral symmetry in the boring apparatus.

The pattern of grooves shows no relationship to the internal ultrastructure or to the crystallographic parameters of the bored substrate. For example, where several tunnels run at different angles within a single calcite crystal, each tunnel has its own set of grooves which are perpendicular to the tunnel axis and are not related to the crystallographic structures of the continuous calcite lattice.

The bored rock material was collected for geological purposes and the samples were only rinsed in distilled water and air dried. Because of this crude treatment, the material
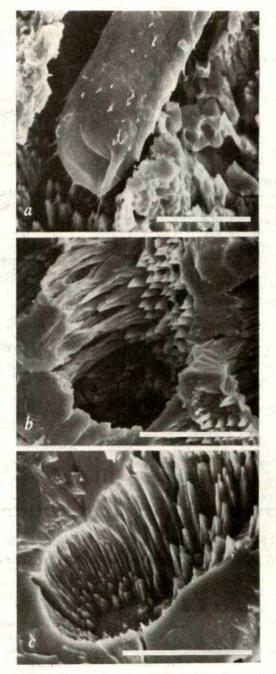
Fig. 1 Cyanophyte borings in calcite: SEM micrographs of fractured material. *a*, Penetrating tip of algal filament, slightly withdrawn from the inner end of the tunnel by shrinkage during preparation (note threads on the filament surface); *b*, oblique fracture cutting four algal tunnels in homogeneous calcite, showing one side of the bilateral pattern of grooves and the zone of small pyramids; *c*, inner end of algal tunnel with grooves and pyramids. Scale bars 10 μm.

is not suitable for a study of soft tissues, and the marks of the boring process cannot be related to specific parts of the algae. Complete algal cells, about 15 μm long and 10 μm in diameter, can obviously be ruled out as direct operators, but identification of possible boring organelles must await properly fixed and dehydrated algal material.

It is, however, interesting to observe that even in this poorly preserved material the algal filaments have a sparse coat of small threads, about 0.2 μm in diameter and 2–6 μm long (Fig. 1a). Similar threads have not been noted in the rich assemblages of endolithic algal filaments found in marine calcareous sediment grains[6,9-12]. Although there is

no real evidence to suggest that the threads are boring apparatus, their presence here is probably significant.

Three conclusions can be drawn from the micromorphology of the borings. First, excavation is a chemical process, but is not straightforward extracellular dissolution on a whole-cell scale. Second external organelles, about 1 μm wide and 10 μm long, are the immediate operators. Third, the boring apparatus has a bilateral symmetry.

It can be added that, although this article considers a single species of *Hormatonema* at one locality only, I have observed similar borings in the course of SEM analyses of sedimentary carbonates from the North Sea and the Baltic Sea. It seems probable that the described mode of boring is common in the cyanophytes.

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Correlation between selenium and mercury in man following exposure to inorganic mercury

THE ability of selenium compounds to modify profoundly the toxicity of both organic and inorganic mercury compounds has been demonstrated in experimental animals by Parizek and co-workers[1,2]. The analytical data of Ganther *et al.*[3] on tuna and of Koeman *et al.*[4] on marine mammals showed that natural levels of mercury and selenium are strongly correlated. Here we report an approximately molar ratio for these elements in certain human organs following exposure to high levels of inorganic mercury.

Mercury in post-mortem samples from three groups of people —workers in the mercury mine at Idrija, Slovenia, inhabitants of the town of Idrija and a control group with no known exposure—has been analysed over the past three years[5,6]. The study has been extended to include selenium. Although we do

not intend to discuss the mercury levels in detail, some interesting findings emerged (Table 1). Notably, the high accumulation and retention of mercury in the thyroid and pituitary; in the miners increases are of the order of a thousandfold, and in the population group about tenfold, relative to the controls. The kidney has previously been considered to be the prime accumulator of inorganic mercury, but in all eight mine workers, it ranked only third. Seven of them had been in retirement for periods up to 16 yr before death (see legend to Table 2), yet the pattern of distribution was essentially similar. It is clear that the retention of mercury in the organs with the highest accumulated levels, namely, thyroid, pituitary, kidney and brain, is very great.

In more recent post-mortem samples we have analysed both selenium and mercury to find a possible relationship between these elements in exposed humans. Both elements were analysed simultaneously by neutron activation from the same sample aliquot, using a volatilisation technique[7] modified to allow separation and measurement of both mercury and selenium[8]. The results to date are shown in Table 2, and clearly demonstrate an approximately 1:1 molar ratio for those organs which accumulate and retain mercury strongly, namely, thyroid, pituitary, and kidney. The same effect is also seen in brain samples (Table 3), with different sections of the same brain all displaying a near molar ratio. Since selenium, as an essential trace element, will normally be present in at least typical physiological levels, whereas mercury in non-exposed persons should only approach insignificant amounts, a molar ratio will only be observed for rather elevated values. Even slightly increased mercury levels, however, seem capable of raising the selenium content; the ratio of the increments over normal levels approaches the molar ratio in many cases.

The correlation coefficient *r* between mercury and selenium in the organ group thyroid, pituitary, kidney and brain (subject T.A.) from the miners is 0.998, with two virtually identical regression equations (*r* very nearly unity) of the form

$$(\text{Se})\text{p.p.m.} = 0.41(\text{Hg})\text{p.p.m.} + 0.21 \ \text{p.p.m.}$$

In molar terms, the ratio Hg–Se is 0.96:1. This ratio in the liver of marine mammals[4] was near 1:1 in molar terms over a 2.5 decade mercury concentration range, but only a small fraction of the mercury could be recovered as methylmercury. In our work, methylmercury levels in the exposed groups, as determined by an isothermal distillation and gas chromatographic method[9], were unremarkable, exceeding normal values only slightly. This also confirms the absence of any significant *in vivo* methylation in man.

The 1:1 molar ratio naturally suggests a direct Hg–Se linkage, though at this stage we can only speculate about the nature of the group or complex and its mode of attachment. Keeping in mind that the time between death and termination of exposure in the professionally exposed subjects varied greatly, it seems that the effect is both accumulative (as in the case of marine mammals and tuna) and retentive. This emphasises the strength of the Hg–Se interaction and suggests their removal from biological turnover. The coaccumulation of selenium observed in this study is certainly not the result of coexisting high levels of

Table 1 Average mercury content with standard deviation of human organs in p.p.m. fresh weight

Group	Thyroid	Pituitary	Kidney	Liver	Lung	Brain
Mercury mine workers	35.2 ± 28.5 (8) 7.8 – 101	27.1 ± 14.9 (7) 13.8 – 64.3	8.4 ± 4.9 (8) 2.3 – 18.5	0.26 ± 0.25 (8) 0.04 – 0.79	1.11 ± 0.89 (5) 0.13 – 1.58	0.70 ± 0.64* (6) 0.18 – 1.50*
Idrija population	0.70 ± 0.45† (10) 0.03 – 3.6	0.46 ± 0.54 (11) 0.02 – 1.76	0.66 ± 1.13 (11) 0.03 – 4.0	0.107 ± 0.059 (11) 0.02 – 0.21	0.127 ± 0.100 (10) 0.005 – 0.24	0.038 ± 0.045† (9) 0.002 – 0.11
Non-exposed controls	0.030 ± 0.037 (16) 0.003 – 0.13	0.040 ± 0.026 (6) 0.007 – 0.064	0.14 ± 0.16 (7) 0.01 – 0.37	0.030 ± 0.017 (8) 0.01 – 0.05	— —	0.0042 ± 0.0026 (5) 0.001 – 0.007

Figures in parentheses refer to the number of subjects analysed, ranges are given below.
* Excluding T.A.; see Tables 2 and 3. † Excluding P.M.

Table 2 Mercury and selenium in human organs in p.p.m. fresh weight

Subject	Thyroid Hg	Se	Hg/Se	Pituitary Hg	Se	Hg/Se	Kidney Hg	Se	Hg/Se	Liver Hg	Se
Mine workers											
F.M. (m)	7.8	3.2	2.4	14.5	6.1	2.4	7.5	2.5	3.0	0.36	0.38
J.V. (m)	29.7	12.6	2.4	13.8	6.4	2.1	6.0	2.1	2.8	0.37	0.40
B.P. (m)	26.5	12.3	2.2	27.7	13.3	2.1	2.3	1.6	1.5	0.037	0.36
T.A. (m)	101	41.1	2.5	47.9	—	—	11.4	5.1	2.2	0.065	0.38
Z.F. (m)	5.0	1.5	3.3								
Idrija population											
P.M. (f)	14.4	5.72	2.5	0.59	0.39	—	0.76	0.70	—	0.067	0.21
D.I. (f)	0.026	0.16	—	0.020	0.34	—	0.61	0.47	—	0.085	0.12
Controls											
D.J. (m)	0.039	0.79	—	0.057	0.45	—	0.37	0.82	—	0.039	0.19
K.H. (f)	0.0023	0.46	—	0.044	0.34	—	0.011	0.54	—	0.011	0.17

Miners: F.M. exposed 17 yr, 5 yr in retirement   B.A. 5 yr in retirement
J.V. exposed 21 yr, 5 yr in retirement   O.A. 10 yr in retirement
B.P. exposed 33 yr, 16 yr in retirement   P.A. 0 yr in retirement
T.A. exposed 29 yr, 16 yr in retirement   L.F. 2 yr in retirement
Z.F. surface worker (welder), 14 yr in retirement
Population: P.M. aged 82 yr, life-long Idrija resident (son a miner)
D.I. 4-month-old infant

Table 3 Mercury and selenium contents of human brain in p.p.m. fresh weight

Sample	T.A. Hg	Se	Hg/Se	B.P. Hg	Se	P.M. Hg	Se
Cerebellum	2.42	1.38	1.8	0.15	—		
	2.34	0.93	2.5				
Striatum and cortex	13.2	6.05	2.2			0.071	0.17
	2.96	1.43	2.1				
Thalamus	8.16	3.33	2.5				
Medulla oblongata	2.66	1.15	2.3				
Hypothalamus				0.10	0.17		
Substantia nigra				0.073	0.17	0.020	0.15
Occipital cortex				0.18	0.23	0.24	0.25

selenium in the mine environment, as analysis of dusts and soils showed low values (around 1 p.p.m.) compared with mercury which is in the 100 p.p.m. range. In marine mammals and tuna the diet is much more enriched in selenium relative to mercury.

Thus in general it seems that the effect can occur where mercury is present as the methyl (tuna) or the inorganic form (marine mammals and man), and whether selenium is relatively abundant in the diet or not. The protective effect of selenium in animals has been shown for various forms of mercury[1,2]. It may be therefore that the coaccumulation of selenium is a natural or autoprotective effect. In this context it is interesting to note that possible preventive and therapeutic uses of selenium against mercury intoxication have been discussed by Parizek[1,2]. Certainly, the detailed metabolic, dynamic and structural mechanisms of this phenomenon seem worthy of further intensive study, in view of its toxicological implications and relevance to the metabolic role of selenium.

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Genetic analysis of behavioural responses to novelty in mice

To trace the genetic determinants of exploratory behaviour in house mice, I started a programme of selective breeding[1]. Using the F_2 generation from a cross between the inbred strains C57BL/6J and DBA/2J as the foundation population, male mice were selected bidirectionally for frequency of exploratory rearing responses displayed in a novel environment. To transfer alleles responsible for high rearing scores from the C57BL strain to the DBA genetic background, selection was combined with repeated backcrossing to DBA females over five consecutive generations. A comparable procedure has been used by Chai[2] for body size in mice. Since these early stages of the selection aimed at developing two lines of mice with similar genetic backgrounds, but differing for loci affecting rearing behaviour, no unselected control line was bred; the DBA strain could serve as this.

In the next stages, selection was continued with concomitant inbreeding by sib-mating to fix the relevant alleles within the two lines. Replicate selected lines[3,4] were not bred. This does not seem too serious a drawback because the purpose of the experiment was to identify individual polygenes controlling exploratory behaviour and the physiological mechanisms through which they act, not to study the potential response to selection *per se*. In the course of this breeding process, a number of the relevant alleles may not be maintained in the lines because of genetic drift (see also Schlager[5], who selected mice for blood pressure level).

After more than twenty generations of inbreeding, the lines, symbolised SRH (high) and SRL (low), must now be close to

Table 1 Raw data for rearing responses and locomotor activity in mice from different generations and genetic analyses under the assumption of monogenic control

Population	n	Mean	Variance	n rating $\geqslant$ critical score* Observed	Expected	χ^2	$P>$
Rearing							
P_1 (SRH)	20	44.25	489.88	16	—	—	—
P_2 (SRL)	20	14.15	42.56	3	—	—	—
F_1	30	32.80	145.48	23	—	—	—
F_2	30	27.47	207.70	16	18.67	1.01	0.30
B_1	30	38.77	201.49	26	23.55	1.19	0.20
B_2	30	24.87	188.12	14	13.80	0.01	0.90
Total†	90	—	—	56	56.02	~0.00	0.99
Locomotion							
P_1 (SRH)	20	95.20	1768.27	17	—	—	—
P_2 (SRL)	20	51.55	773.52	8	—	—	—
F_1	30	50.50	524.12	18	—	—	—
F_2	30	61.90	1200.02	19	18.38	0.06	0.80
B_1	30	71.80	1208.51	21	21.75	0.09	0.70
B_2	30	57.07	1285.99	16	15.00	0.13	0.70
Total†	90	—	—	56	55.13	0.04	0.80

*For rearing, 25; for locomotion, 50.
†$F_2 + B_1 + B_2$.

complete homozygosity and they are still clearly different for rearing score. This is really a difference in the response to novelty, since in a separate experiment with 44 SRH and 46 SRL mice we failed to find any differences between the lines for various exploratory activities carried out in the home cage. The lines seem to differ also for locomotor activity measured in a novel environment[1].

To estimate the number of loci contributing to these behavioural differences, intercrosses (F_1 and F_2) and backcrosses (B_1 and B_2) were made with the two selected lines. This report deals with the results of the analysis of these hybrid types, applying in the first instance methods that have been used with behavioural phenotypes such as susceptibility to audiogenic seizures[6], vocalisation[7], taste sensitivity to PTC[8], saccharin preference[9], and passive-avoidance performance[10] of mice. Single-locus inheritance was indicated and, to confirm this, the next step was a biometrical genetic analysis according to methods described[3-5,11]. The relationship between rearing frequency and locomotor activity was also considered.

Female mice from the SRH strain (20th generation; P_1) were mated with males from strain SRL (20th generation; P_2) to produce an F_1 population (no reciprocals), and the F_2, B_1, and B_2 populations were bred from F_1 females. All neonate animals were fostered to nursing mothers from a random-bred stock, in conditions described previously[1]. At the age of about 13 weeks, single male mice were placed for 15 min during the afternoon in a novel environment, an observation cage measuring $133 \times 53 \times 58$ cm and with a bedding of peat dust. The frequency of rearing responses (vertical activity) and the frequency with which animals intersected light beams activating photocells (horizontal activity) were recorded on counters. The numbers of subjects observed were 20 (P_1, P_2) and 30 (F_1, F_2, B_1, B_2).

The left-hand part of Table 1 shows the untransformed behavioural scores. The non-segregating populations differed from each other for rearing: there was a significant strain difference ($P < 0.001$; Mann–Whitney U test), whereas the F_1 took an intermediate position ($P < 0.03$ and 0.001, respectively). The latter was not true for locomotion; although the parental strains were clearly different ($P < 0.001$), the F_1 differed significantly from the P_1 only ($P < 0.001$) and it resembled the P_2. As far as the distribution of scores is concerned, particularly that of the B_2 suggested bimodality, indicating single-locus control of the behavioural differences. In particular with regard to rearing, the presumptively heterozygous animals within the pertinent populations seemed to have an optimum score (between 25 and 30). On the basis of the numbers of animals from the non-segregating groups that exceed an arbitrary critical score, we can calculate expected values for the segre-

gating populations and compare these with the observed numbers by means of χ-square tests for goodness of fit, as shown in the right-hand part of Table 1. The observed values did not deviate significantly from those expected and, thus, the hypotheses of monogenic inheritance of rearing frequency and of locomotor activity score are not rejected.

A biometrical genetic analysis was also performed; judged by the variances of the raw scores of the non-segregating P_1, P_2, and F_1 populations entered in Table 1, however, significant genotype–environment interactions were present, that is, Mather's second criterion was not fulfilled (F tests; for rearing: $P < 0.001$, 0.005, and 0.005; for locomotion: $P < 0.05$, 0.005, and 0.20). A square-root transformation proved unsatisfactory, but transformation to natural logarithms effectively eliminated the genotype–environment interactions (see Table 2). In these transformed data, significant strain differences were also found for rearing ($P < 0.001$; Student's t test) and locomotion ($P < 0.001$), and the F_1 was again intermediate for rearing ($P < 0.02$ and 0.001, respectively) but not for locomotion (significantly different from the P_1 only; $P < 0.001$). Application of scaling tests A, B, C, and D (Mather's first criterion) in Table 2 shows that the ln transformation rendered the scale of measurement adequate for rearing as well as locomotion, that is, any epistatic interactions were removed, as is also evident from the estimates of components [i], [j], and [l]. With regard to both behaviours, the additivity component [a] was significant and dominance [d] not significant. The heritability, calculated from the components of variation, differed from zero only for rearing (66%) and the minimum estimates of the number of effective loci yielded 0.71 and 0.58, respectively; these values are compatible with single-locus control. Some caution, however, is justified in view of the significant additivity components.

From the correlation coefficients in Table 3 it seems that rearing and locomotion are correlated, even more strongly in the segregating populations than in the non-segregating groups. Significant positive correlations were also established in most of the previous generations of the selection lines; it now seems highly improbable that these resulted from chance fixation of different genes. If traits vary dependently, as shown here for rearing and locomotion, the observed differences

Table 2 Transformed (ln) data for rearing responses and locomotor activity in mice from different generations and results of the biometrical analyses

Population	Rearing Mean	Variance	Locomotion Mean	Variance
P_1 (SRH)	3.70	0.18	4.45	0.24
P_2 (SRL)	2.56	0.19	3.82	0.27
F_1	3.43	0.12	3.80	0.29
F_2	3.17	0.34	3.97	0.38
B_1	3.59	0.15	4.17	0.22
B_2	3.07	0.31	3.83	0.58
Parameter	Estimate	s.e.	Estimate	s.e.
Scaling				
A	−0.05	0.18	−0.08	0.23
B	−0.15	0.23	−0.03	0.32
C	0.44	0.47	−0.01	0.52
D	0.32	0.25	0.05	0.28
Component				
m	2.49*	0.50	4.03*	0.56
a	0.57*	0.07	0.32*	0.08
d	1.78	1.15	−0.003	1.35
i	0.64	0.49	0.11	0.56
j	−0.09	0.28	0.05	0.36
l	−0.84	0.68	−0.22	0.83
Variation				
V_A	0.23		0	
V_D	0		0.14	
V_E	0.12		0.29	
Heritability				
$h^2_{(b)}$	0.66*	0.12	0.36	0.23
$h^2_{(n)}$	0.66*		0	
Effective factors				
k	0.71		0.58	

*Significantly different from zero ($P < 0.001$).

Table 3 Correlation* between rearing responses and locomotor activity in mice from different generations

Population	n	r_s	P
P_1(SRH)	20	0.5564	< 0.01
P_2(SRL)	20	0.6703	< 0.001
F_1	30	0.5540	< 0.001
F_2	30	0.7509	≪ 0.001
B_1	30	0.6987	≪ 0.001
B_2	30	0.7582	≪ 0.001

*Spearman rank correlation coefficients calculated from raw data.

must be the result of variation in the same genetic factor or factors. The locus which controls rearing frequency and the locus which influences locomotor activity may be identical (pleiotropy) or, perhaps, closely linked (gene block).

This report demonstrates that it is possible to single out by means of selective breeding one locus responsible for differences in a quantitative behavioural variable in mice, the reaction to novelty. Previous investigations with the anticholinergic drugs scopolamine and methylscopolamine provided evidence that the genetic factor acts through a hippocampal cholinergic mechanism which regulates exploratory tendencies[1]. The factor may be allelic with the gene *Exa* (for exploratory activity level; identified by using recombinant inbred strains), the action of which can be changed by another locus designated *Sco* (scopolamine-induced modification of exploratory activity[12]). Apart from the isolated gene, other allelic differences that were not maintained during the selection process may also have contributed to the behavioural difference between the ancestral strains.

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'Lesbian' phenotype of *Drosophila melanogaster?*

A BEHAVIOURAL phenotype of female *Drosophila melanogaster* has been found which is characterised by the emission of what seems to be a somewhat rudimentary form of male courtship behaviour; it is directed towards other females, and also towards males. 'Lesbian' behaviour has not been reported before, although it may occur in other strains of *D. melanogaster*.

Female *D. melanogaster* do not usually seem to initiate courtships (although see ref. 1 for factors influencing their attractiveness to males), but take a somewhat passive role—unless they are unreceptive, when a repertoire of rejection responses comes into play[2]. The most important of these rejection behaviours is extrusion of the genitalia towards the courting male.

The courtship of the male has been described several times[3-5]: the male orientates to the female and vibrates one or both wings, producing a signal important in species recognition[6] and in the sexual stimulation of the female[7-9]. The male extends the proboscis and licks the genitalia of the female, at the same time flexing the abdomen in preparation for attempts at copulation. During this procedure the tarsal segments of the forelimbs, with the associated sex combs, are used to grasp the genital region of the female[10].

Females showing lesbian behaviour clearly demonstrate several of these male behaviours. The female orientates to and follows other females, sometimes engaging in a bout of foreleg tapping[3]. Head-on 'courtship' occurs with both wings extended, a relatively rare phenomenon even in males (Fig. 1 *b*). Most of the behaviour is, however, directed to the genitalia of the courted female (Fig 1 *a*), where following and wing extension commonly occur with the tarsi of the forelegs used in a behaviour similar to, but far less precise than, the grasping performed by males; in females it might be called groping. It is not yet known whether an acoustic signal is produced during wing extension, but this behaviour seems to be similar to, although of shorter duration than, the wing vibration of males. In lesbian females it often intergrades into the flicking behaviour[2] of both normal females and males.

Although this behaviour may be performed extremely vigorously, it is broken into discrete bouts of short duration, usually of less than 20 s (mean = 4.68 ± 0.33 s); such bouts may, however, follow one another in quick succession, and on occasions the behaviour becomes effectively continuous. Even in the individuals which show the behaviour most intensively I have not observed proboscis extension and licking of the genitalia; attempted copulation is also absent. A response to lesbian behaviour by sibling virgin females is extrusion of the genitalia; flicking also frequently occurs.

The observations were conducted on pairs of females placed at eclosion in cylindrical plastic cells (30 mm high, 22 mm in diameter), filled to within 1 cm of the top with food medium. Observations were made by repeated scanning of the sample of cells in the evening of days 2 and 3 after eclosion. A female was only classified as of lesbian phenotype when the behaviour had been observed more than once.

Lesbian behaviour has as yet only been found in a series of related 'balanced lethal' strains, designed to maintain the gene *Fs (2)B* (female sterile) in the heterozygous state. In the strain *Fes31*, for example, samples from four different generations gave a mean of 23.7 ± 4.0% females showing lesbian behaviour. The composition of *Fes31* with respect to chromosome II is:

$$fs\ dp^+\ Sp/al^2\ Cy\ (In2LR)\ cn^2\ L^4\ sp^2$$

Lesbian behaviour is not genetically dependent on any of these mutations on chromosome II, since the behaviour is easily retained when both chromosomes II are substituted for those of a laboratory strain in which lesbian behaviour has not been observed (Pacific and Novosibirsk strains).
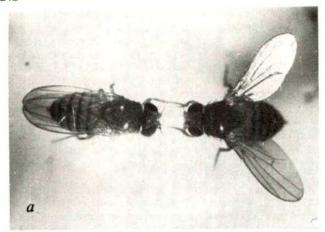
The crossing carried out to transfer the factor(s) responsible for lesbian behaviour to a non-mutant genetical background, rearing in groups rather than single families, has provided the data shown in Table 1. This suggests that *Pacific* is genetically

Table 1 Preliminary genetic data on lesbian phenotype

10 *Fes31* (♀♀ showing lesbian behaviour) × 10 *Pacific* ♂♂		
	Total no.	No. of lesbian females	% Lesbian
F_1	162	17	10.5
F_2	331	98	29.6

10 F_2 (♀♀ from above cross showing lesbian behaviour) × 10 *Pacific* ♂♂		
F_1	74	3	4.0
F_2	72	25	34.7
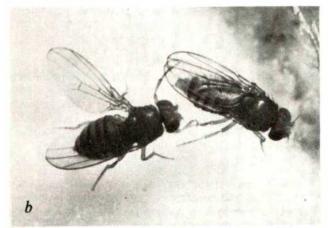
Fig. 1 *a* and *b*, 'Courtship' behaviour of lesbian phenotype females. × 12.

different to *Fes31* with respect to the factor(s) controlling lesbian behaviour, with the *Pacific* allele(s) showing incomplete dominance. Conclusions as to the mode of inheritance of this behaviour must await further genetical and behavioural studies.

Analysis of the data gained from the observations described above shows that lesbian females are found together more frequently than would be expected by assuming a random distribution of such females across the cells (Table 2). In all cases the observed frequencies differ with the same pattern from those expected. It is thus possible that lesbian females mutually stimulate each other into manifestation of lesbian behaviour. This implies that some potentially lesbian females are not detected by the measuring procedure used and that this is therefore an underestimate of the proportion of such females in the sample.

Although this behavioural phenotype closely resembles certain aspects of male courtship behaviour it cannot yet be definitely asserted that it derives from this system. It may, for example, have evolved to serve a function of 'spacing' or 'aggression' between females, or as a response to the abnormal conditions imposed by the mutations of the balanced lethal system.

Whatever its function and origins may prove to be, lesbian behaviour is an item in the (expanding) repertoire of *D. melano gaster* (see for example the 'homosexual' mutant of Gill[11]), on which genetical techniques of analysis may be brought to bear. More detailed behavioural and genetical studies are at present under way.

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Table 2 Statistical analysis of the observed permutations of lesbian / non-lesbian individuals in the observation cells

No. of lesbian flies per cell Strain	No. of cells containing permutations shown		Statistical test
	Expected	Observed values	
Fes31			
2	5.76	12	
1	30.12	18	
0	39.76	46	$\chi^2 = 12.59, P < 0.001$
s/fs (sterile females)			
2	6.20	10	
1	25.80	18	
0	30.02	34	$\chi^2 = 4.82, P < 0.10$
Fes30 × *Novosibirsk*, F_3			
2	4.79	8	
1	16.31	10	
0	13.89	17	$\chi^2 = 5.25, P < 0.10$
Fes30 × *Novosibirsk*, F_4			
2	9.72	21	
1	33.10	16	
0	28.18	34	$\chi^2 = 23.18, P < 0.001$

Entrainment of the pupation and adult activity rhythms during development in the mosquito *Anopheles gambiae*

PREVIOUS work on the initiation of the pupation and flight activity rhythms in mosquitoes[1-3], has been interpreted[4] as showing that there is an ontogenetic distinction between the 'clocks' controlling development and adult behaviour. Results with *Aedes taeniorhynchus*[1,2] suggest that the developmental clock stops after controlling the time of pupation and that the adult behavioural clock does not start until some time after emergence. In *Culex pipiens pallens* the adult clock seems to start just before adult emergence. By contrast, the results reported here indicate that in the mosquito *Anopheles* (*Cellia*) *gambiae* Giles, the flight activity rhythm can be entrained during development at least as early as the second larval stage. The same *Zeitgeber* also sets the phase of the pupation rhythm.
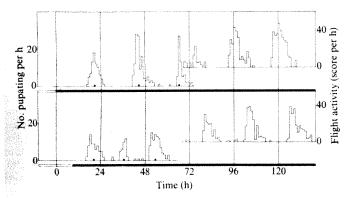
The insects used were *A. gambiae* species A from a culture originating in Pala, Upper Volta. They were reared in LD 12:12 (alternating 12 h light: 12 h dark; 300 lx) and then placed in DD (constant dark) either at the normal time of light-off or after prolonging the light period. Similar experiments with the adults have shown that prolonging the light period resets the flight activity rhythm[5]. In the present experiments 20–40 adults emerged into a muslin cage (30 mm cube) and the total flight activity was monitored using the acoustic actograph technique[6,7]; as in previous exper

ments, a score of 1 was given for any flight activity in any minute. The temperature was maintained at 27 °C.

In the first series of experiments, the change to DD was made during the pupal stage. In an LD 12:12 regime, pupation takes place mainly in the last 6–9 h of the light period, reaching a peak 3–4 h before the end of the light phase. In each experiment, 30–40 pupae, which had pupated between 3.5 and 4.5 h before the normal end of the light phase, were given the final 'light-off' either at the normal time (in this case about 4 h after pupation) or after an extra 12 h of light. In all experiments there was a clear cycle of adult activity which was in phase with the time of the final light-off; the period of the cycle was about 23 h as in previous flight activity experiments[5]. During this series of experiments, adult emergence was monitored under infrared illumination (continued throughout the dark period) using an infrared sensitive TV camera (Link 101S)[x]. The adults emerged 26–30 h after pupation and the time of emergence did not seem to be affected by the difference in the time of light-off. This is consistent with Nayar's observation[1] that the time of emergence in *Ae. taeniorhynchus* is dependent on the pupation rhythm, the duration of the pupal stage being determined mainly by temperature.

In a second series of experiments, the change to DD was made during the larval stage. Figure 1 shows the flight activity of the adults when the change was made during the fourth (final) larval stage and also the timing of pupation in a parallel series of experiments in which mixtures of third and fourth stage larvae were put into constant dim red light. In both series of experiments the change was made either at the normal time of light-off or after prolonging the final light period for 9 h. In the pupation experiments, the pupae were collected from the larval bowls at hourly intervals. In DD (dim red light) there is a cycle of pupation with a period of just under 24 h. Prolonging the final light period does not affect the timing of the first peak, but resetting is complete by the third peak, which seems to correspond to the second peak after light-off at the normal time. Between these two peaks is another smaller peak; this may consist of those individuals which were sufficiently advanced to pupate towards the end of the first reset 'gate'. These results suggest that, if the clock is reset immediately by the extra light, the contribution of the clock to the timing of pupation must take place 24–30 h before pupation actually occurs.

The rhythm of flight activity does not seem to be affected by the actual time of pupation. The flight activity (in DD) of adults derived from pupae selected from these pupation experiments shows that the rhythm is reset by prolonging the final light period, even in individuals which pupate in

Fig. 2 Timing of pupation when the change to DD was made with batches of larvae of different ages.
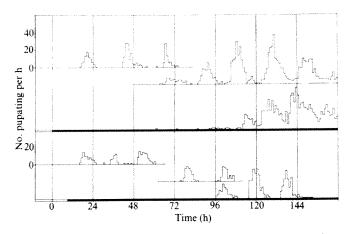
the first peak, in which the time of pupation has been unaffected by the extra light.

Experiments in which the change to DD was made at an increasingly early stage (Fig. 2), indicate that the period of the rhythm controlling pupation remains constant at about 24 h for four cycles and then decreases to 16–20 h. This change in period also occurs when the rhythm is reset by prolonging the final light period. Figure 3 shows the flight activity of mosquitoes which pupated in the peak approximately 90 h after light-off, when the change to DD was made with a mixture of second and third stage larvae. It can be seen that the activity is still cyclical, with a period of about 23 h, and that the phase is determined by the time of light-off. Despite the change in period of the pupation rhythm, mosquitoes which pupated 132 and 144 h after light-off still showed a clear cycle of flight activity, which, in period and phase, was completely consistent with that in Fig. 3.
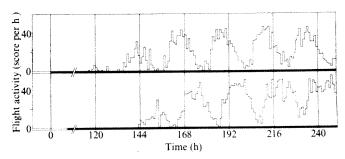
Fig. 3 Flight activity of mosquitoes which pupated approximately 90 h after light-off.

Thus, there seems to be considerable overlap between the 'clocks' controlling the timing of pupation and adult activity and this is surprising in view of findings with other mosquitoes[2,3]. In *Drosophila*[6] the evidence implies that the same clock may 'gate' eclosion and control adult behaviour, persisting through development and metamorphosis. By contrast, in *A. gambiae*, the apparent acceleration of the pupation rhythm, the persistence and stability of the flight activity rhythm and the altering phase relation between the two rhythms suggest that we are dealing with two distinct timing processes. If this is so, the function of the early occurrence of the flight-activity rhythm during development remains a puzzle. Possibly it may play some part in the control of larval or pupal activity.

Fig. 1 Pupation and subsequent adult flight activity after a change to DD late in the larval stage, either at the normal time of light-off or 9 h later. ●, Median of pupation peak.

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Chemotaxis of the spermatozoa of *Ciona intestinalis*

CHEMOTAXIS of animal sperm, long thought not to occur[1-4], was first proved in the marine coelenterate *Campanularia*[5], and since then has been observed in other hydroids[6,8]. The species-specificities and cross reactions between sperm and reproductive structures of these species and genera have been described[6], and some of the attractants have been isolated and their chemical properties reported[7]. In spite of circumstantial evidence for sperm chemotaxis in other phyla[13,14] the sperm behaviour leading to aggregation has not been described. I now report evidence of sperm chemotaxis to egg water and egg extracts (see Table 1 for details) of *Ciona intestinalis* (Protochordata: Urochordata), a primitive chordate. These results imply that sperm chemotaxis either has evolved independently in groups widely separate on the phylogenetic scale or is widespread in the animal kingdom.

Minganti[10] reported species-specificity of the sperm activation and agglutination responses to egg water in five species of Mediterranean tunicates, including *Ciona*. My own observations of *Ciona* sperm in the vicinity of unfertilised eggs indicated that the eggs, or a preparation of egg water (Table 1), activated nearby non-motile spermatozoa[10,11] and seemed to induce turning movements[11]. To investigate whether these turns were chemotactic, *Ciona* gametes were obtained by dissection or by shedding the animals[12] and the methods of observation and photographic techniques previously used on hydroid sperm[5,9] were applied.

Figure 1a shows a typical preparation of *Ciona* sperm swimming against an albumin-coated glass microscope slide under dark field illumination. The cells were describing circles of either 200–250 nm or 100–120 nm diameter. Some cells switched from one diameter to another and often made short, straight deviations shifting the circle to a new position (not shown). In fresh suspensions the diameter of the circle was much larger and some cells followed long, straight paths, possibly because they had not yet made proper contact with the slide surface. As the preparation aged, the straight trails were seen less often or not at all and the diameter of the circles decreased to about 100 nm. The tight circling behaviour sometimes led to the cell leaving the surface and swimming up into the medium.

When seawater was injected from a pipette at the lower left as shown in Fig. 1b, sperm behaviour was basically unaltered. There was no sign of rheotaxis during the 1–2-s injection. When *Ciona* egg extract or egg water was injected (Fig. 1c–f) the cells aggregated rapidly at the pipette tip, sometimes even if no material was injected. After the pipette was removed the aggregate remained but tended to broaden with time. Figure 1f shows that the cells in the aggregate remained motile and did not agglutinate, though some stuck to the slide surface, particularly where the pipette had scraped away the albumin coating.
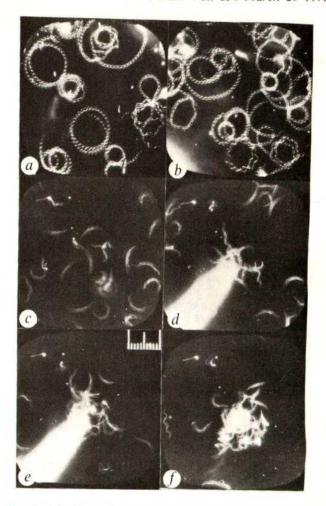
Fig. 1 Behaviour of *Ciona* sperm in the presence of seawater and egg extract injections. *a*, *Ciona* sperm swimming against a glass surface with a beat frequency of 40 Hz. Exposure was for 4 s and flash frequency was 20 Hz. *b*, Similar preparation with a pipette injecting seawater from the lower left, just off the field of view. Exposure was for 2 s and flash frequency was 20 Hz. *c*, Same as (*a*), 0.33 s exposure and flash frequency approximately 40 Hz. *d–f*, Same preparation as (*c*) with a pipette injecting *Ciona* egg extract (*d*, *e*) followed by its removal (*f*). Times between frames are: *c–d*, 2 s; *d–e–f*, 1 s each. The smallest scale division is 10 μm.

To observe the details of sperm behaviour as they entered the aggregation area, individual cells circling on the slide surface were exposed briefly to egg extract released from a nearby micropipette. Figure 2a and c shows the situation before the attractant reached the sperm and Fig. 2b and d shows sperm behaviour during the subsequent few seconds in two typical experiments. When the sperm perceived the attractant they stopped circling and moved rapidly toward the tip of the pipette. If the paths were not straight toward the tip, the cells reoriented by looping (Fig. 2b), by making a second direct turn (Fig. 2d) or by making minor adjustments in the path (Fig. 2b). These behaviours were not seen if seawater was injected in the same situation.

Not all the sperm responded to the attractant, the proportion of non-responders increasing with the age of the preparation. A flagellar beat frequency decrease from 38–41 Hz to 32–35 Hz was associated with loss of responsiveness. In contrast to *Tubularia* sperm[9], it has been difficult to identify a single characteristic flagellar response associated with turning. Furthermore, preliminary measurements indicate no marked increase in swimming velocity as the sperm moved towards the source of attractant in spite of small adjustments of beat frequency like that shown in Fig. 2d. This latter observation may, however, indicate merely that the sperm were already swimming at the maximum velocity possible.

Table 1 Specificity of urochordate sperm chemotaxis
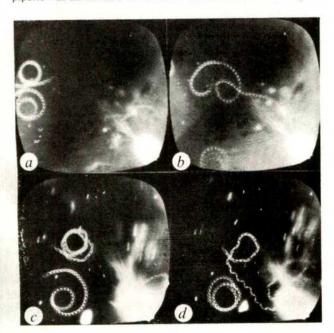
Sperm type Egg extract (titre)	*Ciona*	*Styela plicata*	*Styela clava*	*Tubularia* (Hydrozoa)
Ciona (9)	+ (> 100)	− (10)	− (4)	− (5)
S. plicata (4)	− (10)	+ (8)	NT	− (2)
S. clava (0)	− (4)	NT	− (4)	NT
Tubularia (12)	− (5)	− (2)	− (2)	+ (> 100)

Numbers in parentheses in columns 2–5 indicate numbers of trials. +, Aggregation of motile sperm; NT, not tested. Egg water was prepared by placing several thousand prewashed eggs into 5 ml of seawater and leaving the preparation to stand for 3–5 h at room temperature. The medium was then drawn off the eggs and tested against fresh sperm. Eggs treated in this manner are fertilisable and develop into normal tadpoles. Egg extract was prepared by removing the seawater from several thousand eggs and replacing it with a small volume of 100% ethanol. After a suitable extraction period (30 min at room temperature) the alcohol was drawn off, evaporated to dryness and the residue was diluted into seawater for testing. One hundred *Ciona* eggs, extracted in 50 µl of ethanol, produced an active seawater solution which could be half-serially diluted eight or nine times before the attracting activity was lost (titre = 8–9). Five times this number of eggs of either species of *Styela*, extracted in the same manner, gave solutions with no activity against *Ciona* sperm. Follicle cells were isolated by passing eggs through a Pasteur pipette several times in calcium–magnesium-free seawater. The eggs were removed by low speed centrifugation (500 r.p.m.) and the follicle cells were collected at 2,000 r.p.m.

Although attractant activity can be obtained by alcoholic extraction of several tissues of adult *Ciona* (particularly those associated with the ovotestis) eggs consistently produced the highest titres. The distribution of attractant activity in adult tissues remains to be determined. Extracts of tissues from immature *Ciona* were inactive. Extracts of isolated outer egg follicle cells were very active and the eggs from which they had been removed remained active (see Table 1 for method). Since all the follicle cells could not be removed from the egg chorions, and since both egg and follicle cell preparations contained a clear, viscous mucus as a persistent contaminant, the exact source of the attractant remains in doubt.

The species-specificity of the response has been partially determined. Alcohol extracts of eggs of the tunicate *Styela plicata* obtained by light-induced shedding (C. Lambert, personal communication), had no effect on *Ciona* sperm, but they did attract sperm of *S. plicata*. Extracts of *Ciona* eggs of rather high titres (Table 1) had no effect on the sperm of *S. plicata*. No chemotaxis has been observed in the sperm of *Styela clava* toward any of the egg extracts used, even those prepared from eggs of this species. There is no cross reactivity between the sperm and sperm attractants of

Tubularia and *Ciona* (Table 1). Curiously, although *Ciona* is known to be largely self-sterile[13], egg extracts prepared from any one animal will attract sperm obtained from that same animal.

Observations that chemotaxis of the sperm of the hydroid *Hydractinia echinata* occurs to eggs of that species[11] have confirmed directly the ability of spawned eggs, free of complex outer investments, to attract spermatozoa species-specifically. *Ciona* also sheds its eggs before fertilisation, and though the egg bears a complex array of investing cells, it nevertheless produces a substance capable of attracting sperm species-specifically. Therefore, arguments[16,17] that fertilisation of animal eggs in an aqueous medium occurs as the result of chance encounters with sperm may have to be re-evaluated. A possible advantage of sperm chemotaxis to spawned eggs comes from the effective increase in volume of the egg as a target for the sperm. Its further role in animal fertilisation remains to be determined.

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Fig. 2 Sperm attraction by *Ciona* egg extract. *a* and *c*, Two cells circling on a glass slide close to a pipette injecting egg extract (beat frequency approximately 40 Hz). Exposure: *a*, 2.5 s; *c*, 1.5 s; flash frequency was 17 Hz. *b* and *d*, Less than 1 s later. Exposure: *b*, 3 s; *d*, 2.25 s; flash frequency was 17 Hz. *b*, Response of the lower of the two cells in (*a*). The change in beat frequency for the upper cell in (*d*) as it approached the pipette was an increase of about 1.5 Hz. Same scale as in Fig. 1.

Different drugs arrest cells at a number of distinct stages in G₂
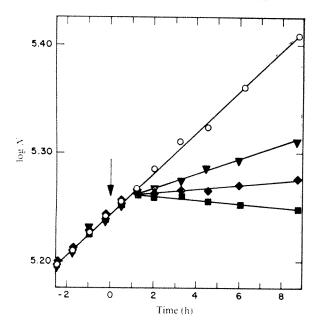
SEVERAL physical and chemical agents can induce susceptible mammalian cell populations to accumulate in the G_2 phase of the cell cycle[1-8]. The question arises whether cells treated with different agents all accumulate at a single 'restriction' stage within G_2 or, alternatively, stop at a variety of stages.

Techniques of cell-cycle analysis can show the precise

stage at which cycle progression capacity is lost in cells treated with inhibitory substances. If a drug is added to an exponentially growing culture, the time between addition and change in growth rate determines the time in the cell cycle preceding division—the M/G_1 boundary—at which the agent inhibited progression[9]. Cells at a stage beyond this temporal marker at the time of addition continue through the cycle and divide, while those at earlier stages are prevented from progressing at the normal rate. The time is calculated back from M/G_1, since division is the parameter measured.

To determine the precise stage in G_2 that selected chemotherapeutic agents stopped cell-cycle progression, I measured the concentration of cells in exponentially growing cultures of CHO Chinese hamster cells at different times before and after addition of drugs. Figure 1 shows data obtained by treating cells with various concentrations of neocarzinostatin, a compound effective against human leukaemias[10]. Although the exact mode of action for this drug is unknown, neocarzinostatin apparently interacts with the DNA, causing a change in DNA structure[11]. The cells continued dividing at the exponential growth rate (culture doubling time of 16.5 h) for 60 min after addition of the drug; thereafter, in cultures receiving large concentrations of the drug, there was no further increase in cell number, and there was even an indication of a slight loss in the number of cells. With lower concentrations, where the cell cycle was incompletely inhibited, the rate of increase in cell number was decreased, commencing 60 min after addition of drug. Although the rate of progression for affected cells was different at the three drug concentrations, a value of 60 min was obtained in all three cultures for the time interval between addition of the drug and change in the rate of division. Therefore, with a wide (20-fold) range of drug concentrations, the terminal point of action of neocarzinostatin within G_2 appeared to be independent of concentration. (A similar effect was obtained with the other agents studied.) Continued incubation of these cultures for 36 h, in the presence or absence of drug (that is, cells resuspended in drug-free medium after treatment with neo-

Fig. 1 Determination of the terminal point of action of neocarzinostatin in line CHO Chinese hamster cells. A culture of exponentially growing CHO cells in F-10 medium, supplemented with 10% calf serum and 5% foetal calf serum, was split into four subcultures. At time zero, one culture received no drug and served as the control (○). Neocarzinostatin was added to the other cultures, yielding final concentrations of 50 µg ml⁻¹ (▲), 400 µg ml⁻¹ (◆), or 1,000 µg ml⁻¹ (■). Cells were counted at hourly intervals with an electronic cell counter.
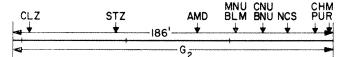
Fig. 2 Drug-specific arrest of progression at different stages within the G_2 phase of the cell cycle in asynchronous cultures of CHO cells. The terminal point of action was determined as in Fig. 1 for the following agents: CLZ, chlorozotocin, NSC No. 178248; STZ, streptozotocin, NSC No. 85998; AMD, actinomycin D, NSC No. 3053; MNU, 1-*trans*-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Me-CCNU), NSC No. 95441; BLM, bleomycin, NSC No. 125066; CNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), NSC No. 79037; BNU, 1,3-*bis*-(2-chloroethyl)-1-nitrosourea (BCNU), NSC No. 409962; NCS, neocarzinostatin, NSC No. 69856; CHM, cycloheximide, NSC No. 185; and PUR, puromycin, NSC No. 3055. Drugs were obtained through Drug Research and Development, Division of Cancer Treatment, National Cancer Institute. The drug solutions were prepared immediately before use. The terminal point of action for each agent was determined in multiple cultures using 20–80-fold concentration ranges for each drug.

carzinostatin for 1 h), resulted in populations comprised almost entirely of cells in G_2, based on measurements of population DNA content obtained with the Los Alamos flow microfluorometer[8]. Thus, there was a net increase in cells in G_2, indicating that the cells initially in late G_2, M, G_1 or S could reach G_2, but no further. Some irreversible change occurred in response to treatment with neocarzinostatin that predisposed the cells to stop in G_2, regardless of the stage of the cell cycle in which they resided while exposed to the drug.

In similar fashion, the terminal point of action was determined for various drugs which cause cells to accumulate in G_2, as shown in Fig. 2[8,12,13]. The agents tested caused the cells to stop in different portions of G_2. Thus, at least in so far as the agents studied are concerned, cells do not stop at a single stage within G_2. Rather, the data seem to agree well with the model proposed by Mitchison[14] for multiple transition points within G_2.

With the exception of the protein synthesis inhibitors (puromycin and cycloheximide), all agents tested caused at least a partial enrichment in the fraction of cells in G_2, similar to the effects of neocarzinostatin. Although accumulation of cells in G_2 was possible with these agents, the effects on progression capacity were not readily reversible, so that none of these agents seem to be useful in preparing synchronised populations for study of late interphase events.

I have not devised a testable model to encompass all the observations reported here. The well documented arrest of mammalian cells in G_2 after X-irradiation, however, shares many features with the results presented here[1–4]. Perhaps the consequences to the genome of X-irradiation and treatment with these agents are similar in mammalian cells[15,16], although the actual biochemical mechanisms may be different.

I should like to hypothesise that a surveillance mechanism operates throughout G_2 to eliminate from the proliferative mode cells with altered DNA. Such a surveillance mechanism would examine cells at multiple stages within G_2 to ensure that either the damaged DNA was repaired normally, in which case a cell would be returned to cycle, or an unrepaired cell would be converted to a non-viable state, even though several abortive rounds of division might occur before cell death. (From an evolutionary standpoint, conversion to a nonviable state would limit the dissemination of grossly deranged mutant cells and would decrease the frequency of survival of the damaged cell in competing with normal cells for essential nutrients.) Thus, in this model, G_2 would represent a crucial period during which an essential decision is made concerning further cellular proliferation. This model does not exclude the possibility that

additional proliferation decisions could be made in other portions of the cell cycle.

The hypothetical surveillance mechanism is not foolproof, since viable mutants have been isolated from mammalian cell lines[17,18]. Of course, a totally foolproof mechanism would preclude even evolutionary changes. I feel that drugs such as we used may provide valuable probes for future studies of proliferation control mechanisms.

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Induced agglutinability of 3T3 mouse fibroblasts

CONCANAVALIN A (con A) preferentially agglutinates polyoma virus-transformed 3T3 cells (Py3T3) compared with 3T3 cells[1]. Brief proteolytic treatment of 3T3 cells results in agglutinability comparable with that of Py3T3 cells, and this is consistent with the suggestion that constraints at the 3T3 cell surface prevent migration and clustering of con A receptors[1-3]. We now report that 3T3 cells are as equally agglutinable as Py3T3 cells after a brief exposure to low ionic strength conditions (LIS, isosmotic). The data suggest that the mobility of con A receptors is altered by electrostatic interactions at the cell surface.

Py3T3 and 3T3 cells were cultured on plastic substrate at 37 °C, pH 7.0, in Dulbecco's modification of Eagle's MEM, supplemented with 10% foetal calf serum and antibiotics (Grand Island Biological Co.). Preliminary experiments showed that agglutination by con A was a function of time, pH, con A concentration, cell density and oscillations per min for the reaction vessels[4]. Osmolarity was maintained at 340 mosmol for all assays using D-mannitol, which does not inhibit con A binding[5]. Hapten-inhibition with α-methyl-D-mannoside was used to confirm con A specificity. The con A was grade III, twice crystallised (Sigma).

Some properties of Py3T3 and 3T3 cells, determined in this laboratory are shown in Table 1. Half-maximal agglutination (HMA, defined as the concentration of con A required to agglutinate 75% of the cells) was 650 µg ml^{-1} for 3T3 cells compared with 75 µg ml^{-1} for Py3T3, when assayed at regular ionic strength (RIS, 150 mM). Prior incubation of 3T3 cells for 30 min at LIS in the presence of con A increased the agglutinability considerably (Fig. 1). The HMA was shifted to 75 µg ml^{-1}, and the concentration dependence was similar to that observed for Py3T3 cells at RIS. This

Table 1 Properties of mouse fibroblast lines used

	3T3	Py3T3
Generation time (h)	23.5 ± 0.1	29.1 ± 0.6
Saturation density (cells cm^{-2})	6.4×10^4	86.0×10^4
Transplantability	Negative	Positive
Agglutinability (HMA)		
RIS (150 mM)	650	75
LIS (< 50 mM)	75	75

Transplantability was tested by injecting 20 Swiss albino mice (immunosuppressed using 450 rad, whole body) with 10^7 viable cells from logarithmic-phase cultures. Py3T3 cells caused progressive tumour growth in 17 out of 20 mice, compared with 0 out of 20 when injected with 3T3 cells. All agglutination assays used 5×10^5 cells per ml, collected from log-phase cultures, rinsed twice with phosphate-buffered saline (PBS) containing 5 mM EDTA, followed by suspension in PBS containing 10 mM CaCl$_2$. Con A was added using constant volumes, 0.5 ml cells and 0.5 ml con A, incubated for 30 min at 37 °C, and 125 Hz min^{-1}. HMA is defined as the concentration of con A (µg ml^{-1}) required to agglutinate 75% of the cells. LIS condition was a 30 min incubation before addition of con A. Control agglutination (no con A) did not exceed 10%. Percentage of cells agglutinated was calculated by multiplying average clump size by number of clumps compared with free cells.

enhanced agglutination was observed once the ionic strength was below 50 mM (Fig. 2).

The following properties of this induced agglutinability were demonstrated in parallel experiments: (a) enhanced agglutination was only observed when con A was present with the cells at LIS, that is, incubation at LIS without con A, followed by addition of con A and assay at RIS did not enhance agglutination; (b) once cells were incubated with con A at LIS, agglutinability was enhanced when assayed either at LIS or RIS and (c) the LIS effect was not altered by removal of sodium, potassium or chloride ions (shown by holding the ionic strength constant using other monovalent salts).

Table 2 summarises the nature of the LIS effect with an attached cell preparation using human erythrocytes[6]. The LIS effect was temperature-sensitive, con A-specific, blocked by prior fixation of the cell membrane and not affected by inhibitors of metabolic energy.

These data demonstrate the importance of the ionic environment at the cell surface in determining agglutinability. The enhanced agglutination of 3T3 cells at LIS was phenomenologically identical to the agglutination of Py3T3 cells at RIS by the following criteria: (a) the LIS effect was abrogated using an inhibitor of con A binding; (b) the effect was temperature-sensitive and unaffected by inhibitors of metabolic energy; (c) the HMA was the same value at LIS

Fig. 1 The concentration dependence of agglutination with con A is shown for Py3T3 cells at RIS (150 mM) (– – – –); 3T3 cells at RIS (●); and 3T3 cells at LIS (< 50 mM) (○). Agglutination assays performed as described in Table 1 and values represent the means of triplicate determinations.
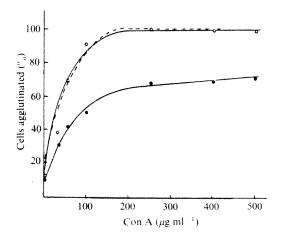
Table 2 Effect of various treatments on enhanced agglutinability of 3T3 cells at LIS

Treatment	Effect on agglutination
37 °C	Enhanced agglutination
25 °C	Enhanced agglutination
4 °C	No agglutination
Formalin (10%, 30 min)	No agglutination
Glutaradehyde (2.5%, 30 min)	No agglutination
2,4-Dinitrophenol (10 mM)	Enhanced agglutination
Sodium azide (10 mM)	Enhanced agglutination
Potassium cyanide (10 mM)	Enhanced agglutination
α-Methyl-D-mannoside (50 mM)	No agglutination

Cultures were rinsed with PBS, pH 7.2, and incubated at LIS with 340 mM D-mannitol, buffered to pH 7.0 with 10^{-5} M phosphate. Haemadsorption assay used human type O erythrocytes[12] layered over the attached cells at a 1% concentration for 30 min, then removed by gentle rinsing with PBS. Results shown above used monolayer 3T3 cells treated with con A (50 μg ml^{-1}) after the indicated cell treatment. Enhanced agglutination indicates heavy clusters (> 10 per cell) of erythrocytes attached to 3T3 cells; no agglutination indicates background adsorption (0–3 per cell). Similar results were obtained using erythrocytes treated with con A, then assayed against untreated 3T3 cells.

for 3T3 cells as observed for Py3T3 cells at RIS and had the same concentration dependence, and (d) it was not necessary to digest the cell membrane with proteolytic enzymes to enhance agglutinability.

The above observations are consistent with the following concept of electrostatic interactions at the cell surface on addition of con A. Lipid bilayers are sensitive to the ionic environment[7,8], and binding con A results in an increased surface charge density[9]. Sufficient instability of lipid may require the reorganisation of lipid structure (to maximise order and decrease surface charge density) resulting in the
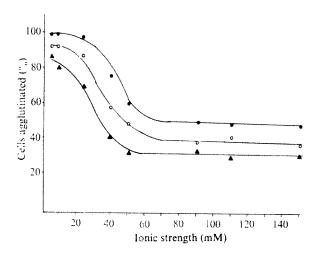
Fig. 2 Agglutination of 3T3 cells as a function of ionic strength. Con A at 200 μg ml^{-1} (●), 100 μg ml^{-1} (○), and 50 μg ml^{-1} (▲).

migration and clustering of con A receptors. Clustered binding sites could be stabilised due to cross-linking by con A. The threshold for lipid instability is considered low for 3T3 cells compared with Py3T3 cells since the membrane lipid is inherently more fluid in the transformed cell[10]. A low threshold for 3T3 cells is obtained by incubation at LIS, which increases the surface charge density[11], and addition of con A causes threshold instability resulting in the clustering of con A receptors. Although other possibilities exist, the concept of a threshold electrostatic condition for the instability of membrane lipid is consistent with the inherent

differences in agglutinability of Py3T3 and 3T3 cells, and enhanced agglutination at LIS.

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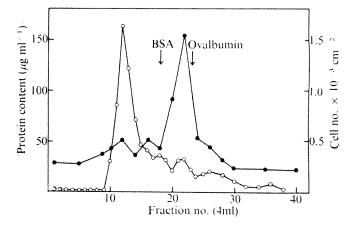
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Growth-enhancing protein obtained from cell surface of cultured fibroblasts

NORMAL animal cells do not grow at extremely low cell density under ordinary culture conditions[1,2]. This property of population dependence on cell proliferation has long been a crucial disadvantage for establishing clonal cultures of animal cells. Feeder layers or conditioned media are therefore used to sustain the growth of single cells in culture. The growth-enhancing material in conditioned media, the conditioning factor, is thought to be a large aggregate of macromolecules of pericellular environment[2]. This assumption was confirmed by the findings that cultured chick embryo fibroblasts laid down a microexudate carpet on the surface of Petri dishes and that this carpet exerted a growth-enhancing activity on sparsely seeded fibroblasts[3,4]. Although the microexudate was thought to be composed mainly of glycoproteins, its exact chemical nature has not been known[3-5]. Here we report the solubilisation and purification of a growth-enhancing protein from the microexudate of cultured chick embryo fibroblasts.

Kondo and Sakai[6] extracted a cell aggregation factor by dissociating sea urchin embryos with 1 M urea–5 mM EDTA solution and showed that this factor was precipitated in the presence of calcium ions. This method was used here for the extraction and purification of the exudate of cultured fibro-

Fig. 1 Purification of the growth-enhancing protein on a column of Sephadex G–200. Calcium precipitate obtained from 100 Petri dishes (10 mg protein) was dissolved in 1 ml of 6 M urea–10 mM EDTA at pH 8.0, stored at 4 °C for 2 weeks and then loaded on a column of Sephadex G–200 (1.5 × 75 cm). Elution was carried out with 10 mM Tris–HCl buffer containing 0.1 mM EDTA at pH 7.5 and fractions of 4 ml were collected. Aliquots of 0.2 ml were used to determine the growth-enhancing activity on sparse cell cultures in the same way as described in Table 1. The arrows indicate the positions of bovine serum albumin and ovalbumin used as the standards.

blasts. Monolayer cultures of chick embryo cells were prepared from 9 or 10-d-old chick embryos in Eagle's MEM supplemented with 5% foetal bovine serum. Cells were cultivated for 3–4 d until they reached confluence in 10-cm Petri dishes. Media was discarded, cell layers were washed twice with phosphate buffered saline containing 0.1 mM EDTA and incubated with 5 ml of 10 mM Tris buffer containing 1 M urea and 5 mM EDTA (pH 8.3) at 37 °C for 30 min or at room temperature for 2 h. The cells became round and tended to become detached from dishes, and they were then dissociated from the dishes by gentle pipetting. More than 99% of cells were viable as judged by staining with trypan blue. After removal of cells and debris, the extract from 100 dishes was concentrated to 50 ml with a Diaflo PM–10 membrane. The crude extract showed strong growth-enhancing activity when added to sparsely seeded cell cultures (Table 1, experiment *a*). The maximum growth-enhancement was obtained by 40 μg ml^{-1} of the crude extract, but the extract at higher concentrations was somewhat inhibitory to the cell proliferation. This effect was caused by a macromolecular growth inhibitor separated by subsequent purification (unpublished work). Quantitative assay of the growth-enhancing activity was difficult because the assay depended strictly on the inoculum density and physiological condition of the cells and because of the existence of inhibitory substances. The degree of subsequent purification, however, could be estimated roughly in terms of the doses required for maximum enhancement.

Table 1 Growth-enhancing activity of the crude extract and calcium precipitate on cultured chick embryo fibroblasts

Experiments	Dose (protein content μg ml^{-1})	Stimulation of growth (Cell no. × 10^{-3} cm^{-2})
a, Control		2.0 (±0.3)
Crude extract	2	7.9 (±2.3)
	10	15.8 (±3.1)
	40	22.3 (±2.4)
	80	9.3 (±2.0)
b, Control		0.7 (±0.2)
Calcium precipitate	2	2.6 (±0.2)
	5	2.9 (±0.4)
	10	4.6 (±0.2)
	20	3.7 (±0.3)
Calcium–supernatant	5	0.8 (±0.1)
	10	0.8 (±0.1)
	20	0.5 (±0.2)

All samples were dialysed against 10 mM Tris–HCl buffer containing 0.1 mM EDTA at pH 7.5 before the determination of the growth-enhancing activity. Aliquots of 0.02 to 0.2 ml of samples were sterilised in 3.5 cm Falcon plastic Petri dishes by brief ultraviolet irradiation (at a distance of 30 cm from a 15 W germicidal lamp for 5 min). Cultivation was started at a density of 500–1,000 cells cm^{-2} in 2 ml of Eagle's MEM containing 5% foetal bovine serum, and the numbers of cells were counted after 7 d. At these low cell densities, the growth rate in the control plates was very low[3]. All experiments were done in duplicate. Protein content was determined by the method of Lowry *et al.*[10].

The concentrated extract was dialysed against 10 mM Tris–HCl buffer containing 10 mM calcium chloride at pH 7.5. The white precipitate which appeared in a dialysing tube (about 25% of total protein) was collected by centrifugation and dissolved in 6 M urea–10 mM EDTA at pH 8.0. The activity was found only in the calcium-precipitate fraction (Table 1, experiment *b*). Since this fraction showed maximum enhancement at 10 μg ml^{-1}, it was concluded that almost all the activity was recovered in the calcium precipitate.

The calcium precipitation was repeated again and the precipitate (about 20% of total protein in the crude extract) was dissolved in 1 ml of 6 M urea–10 EDTA at pH 8.0. After storage at 4 °C for two weeks, the materials were fractionated on a column of Sephadex G–200. The growth-enhancing activity was eluted as a single peak, between the positions of bovine serum albumin and ovalbumin (Fig. 2). This peak contained a single protein as shown by polyacrylamide gel electrophoresis

a   *b*
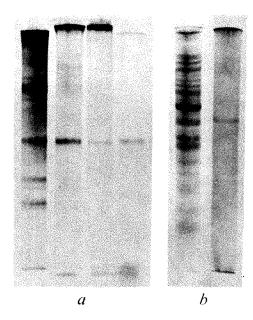
Fig. 2 Patterns of polyacrylamide gel electrophoresis with or without SDS at each purification step. *a*, Electrophoresis in 7.5% gels without SDS at pH 8.1 (ref. 11). From left to right; crude extract, first calcium precipitate, second calcium precipitate, and purified protein. *b*, SDS–polyacrylamide gel electrophoresis in 10% gels[12]. Left, crude extract; right, purified protein. The molecular weight of the purified protein was estimated to be 48,000 (mean of several experiments) using bovine serum albumin, ovalbumin and lysozyme as the standards.

with or without SDS (Fig. 3), of molecular weight about 48,000. This value agreed with the results of Sephadex G–200 column chromatography (Fig. 2). About 400 μg of purified protein (1.5% of total protein in the crude extract) was obtained from 100 dishes. Prolonged treatment of the calcium precipitate in urea–EDTA solution was necessary to dissociate the active materials into monomers of molecular weight 48,000. When this treatment was reduced, two additional peaks of activity were usually found after fractionation on Sephadex G–200, one corresponding to a molecular weight of about 100,000 and the other at the void volume position. In these procedures, it should be also noted that the abrupt addition of calcium chloride often caused a drop in the pH, resulting in the coprecipitation of inactive materials.

Figure 4 shows the effect of purified protein on the growth rate of cultured fibroblasts seeded at low cell density. The purified protein exerted its maximum growth-enhancing effect at 1 to 2 μg ml^{-1}, and the activity was lost completely by treatment with 0.1% trypsin at 37 °C for 30 min. Primary cultures of mouse embryo cells was also stimulated. The activity was retained after brief ultraviolet sterilisation, after treatment with urea or several kinds of detergents, and after freezing–thawing or storage of the solution at 4 °C for at least 2 months. The protein tends to precipitate at a pH lower than 5.0 or in the presence of a small amount of calcium or magnesium ions. Although preliminary experiments indicated that radioactive glucosamine was incorporated into this protein, chemical compositions have not yet been determined because of low yields.

The present investigation has proved that urea–EDTA solution[6] is also useful for the extraction of cell surface or extracellular materials of higher animals with minimum destruction of cells, because 1 M urea solution is nearly isotonic with the cells. We have isolated the same growth-enhancing protein from chicken embryonic tissues by the direct extraction with 1 M urea–5 mM EDTA solution (Y.Y., unpublished).

As expected previously[1–4], the purified protein has properties characteristic of pericellular structural proteins, indicating that this protein is responsible for the growth-enhancing activity of
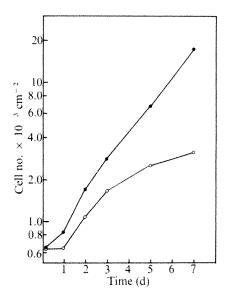
Fig. 3 Effect of purified protein on the growth rate of cultured fibroblasts at low cell density. ○, Control; ●, 1 µg ml^{-1} of the purified protein was added at the start of the cultivation.

conditioned media or feeder layers. Calcium ions may be required for the maintenance of the structural integrity and growth-enhancing activity of the microexudate carpet. In this respect, the carpet protein should be distinguished from humoral growth regulators such as hormones, serum components or fibroblast growth factor[7]. Apparently it resembles closely the "mesenchymal factor" which was partially purified from chick embryonic tissues by Ronzio and Rutter[8] with respect to its structural protein nature, the molecular weight of the monomer, trypsin-sensitivity, possible contribution of calcium ions for activity, and in its localisation at cell surfaces[9]. These factors may offer a molecular and morphological basis for understanding the contact or short range interactions affecting the proliferation of animal cells, with special reference to the functions of cell surfaces.

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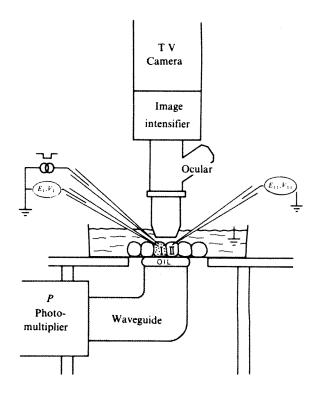
Permeability of cell junction depends on local cytoplasmic calcium activity

MANY kinds of cells are coupled by junctions consisting of membrane channels through which molecules of a certain size range can flow freely from one cell interior to another[1]. It has been proposed that the permeability of the junctional channels depends on the concentration of free ionised calcium in cytoplasm ($[Ca^{2+}]_i$) (ref. 2). This hypothesis is supported by two classes of experiments. In one, the interior of a coupled cell system is allowed to exchange freely with a known $[Ca^{2+}]$ in the exterior, through a hole in the (non-

junctional) membrane; junctional conductance is reduced (uncoupling) when the $[Ca^{2+}]_i$ is above $5{-}8 \times 10^{-5}$ M (ref. 3). In the other class, uncoupling ensues when the (closed) cell system is treated with inhibitors of energy metabolism or with Ca^{2+} ionophores[5], or on exposure for long periods to Ca,Mg-free medium or to Li medium[6]; in these conditions a rise in $[Ca^{2+}]_i$ may be expected because of known properties of cellular Ca metabolism[7,8]. Here, we demonstrate the changes in $[Ca^{2+}]_i$ together with those in coupling in three of these conditions, using aequorin to display the distribution of Ca^{2+} in the cell. It will be shown that the uncoupling is, indeed, in each case associated with a rise in $[Ca^{2+}]$. Furthermore, by local injection of Ca^{2+} into the cells, it will be shown that uncoupling ensues when the rise in $[Ca^{2+}]_i$ occurs at the junction, but not when it occurs at other regions in the cell.

Chironomus salivary glands were isolated in physiological medium as described previously[4], except that the medium contained 12 mM Ca and no Mg. Purified aequorin was injected into one or two adjacent cells. This protein reacts with Ca^{2+}, emitting light. The emission, approximately proportional to $[Ca^{2+}]^2$ (ref. 9), provides a convenient $[Ca^{2+}]_i$ indicator[10–12]. The aequorin had no apparent adverse effects; membrane potentials and coupling were maintained for several hours. Light emitted inside the cells was guided by a light pipe to a photomultiplier. In addition, a television camera coupled to an image intensifier viewed the cell luminescence through a microscope (Fig. 1). This novel aspect of aequorin technique enabled us to see where the light was emitted inside the cells with a resolution of 5 µm. Ca buffered with EGTA (to stabilise the $[Ca^{2+}]$) was pressure-injected into the cell containing the current source, while electrical coupling between this cell and a contiguous cell was measured as shown in Fig. 1. Current pulses, membrane potentials, their displacements, and photocurrent

Fig. 1 Television camera coupled to image intensifier by a light guide views the aequorin luminescence of the cells through microscope (darkfield); luminescence is also measured by photomultiplier. Electrical coupling is measured with the aid of three microelectrodes, by pulsing current (i) between the inside of cell (I) and the outside and measuring the resulting steady-state changes (V) and membrane potentials (E) in I and adjacent cell II.
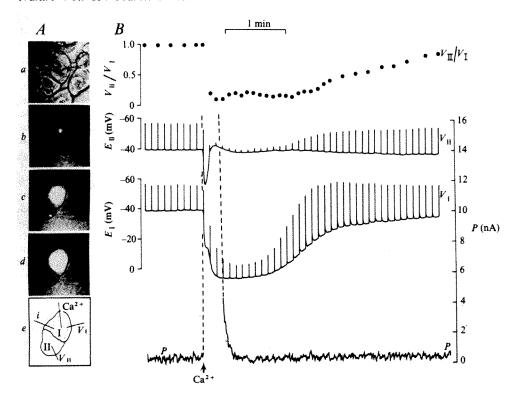
Fig. 2 Ca^{2+} injection. *A*, Darkfield television pictures (*b-d*) of aequorin luminescence in a cell, produced by three puffs of 5×10^{-5} M free Ca (buffered) of increasing magnitude, delivered to cell centre. The pictures are videotape photographs each taken at the time of maximum luminescence spread. Cell outlines are traced by superposition of brightfield video picture (*a*); cell diameter ~ 100 μm. Puffs *b* and *c* do not reach the junctions of the cell and do not affect coupling. Puff *d* reaches one junction and causes transient uncoupling, as shown in *B*: chart records of photomultiplier current P, E_I, E_{II}, V_I, V_{II} ($i = 4 \times 10^{-8}$ A); and plot of coupling ratio V_{II}/V_I. *e*, Cell diagram showing locations of microelectrodes and of Ca injection micropipette; dotted cell preinjected with aequorin.

were displayed on a chart recorder and on a storage oscilloscope on to which a second television camera was focused. The two camera outputs were displayed together on a monitor and videotaped. Thus, we had continuous and simultaneous information on the electrical parameters and on the relative local Ca^{2+} activities inside the cells.
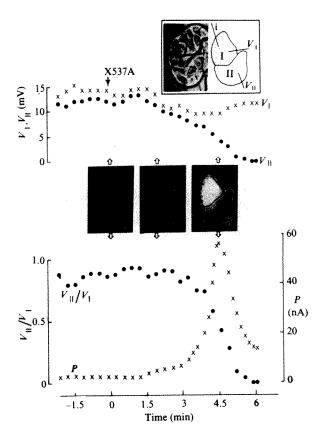
Short single injection pulses of $5-10 \times 10^{-3}$ M free Ca^{2+} into healthy cells produced aequorin glows that were confined to the immediate vicinity of the tip of the injection pipette (Fig. 2). Evidently the Ca^{2+} is prevented from diffusing freely through the cytoplasm and seems to be sequestered by intracellular elements[7,13,14]. When such an injection was made into the cell centre (cell radius, 50 μm), junctional coupling was unaffected. When it was close to a cell junction, the coupling fell (uncoupling), and this effect was confined to the junction at which the local rise in $[Ca^{2+}]$ had occurred. Longer or more frequent injections of this sort, or prolonged Ca^{2+} iontophoresis between electrodes placed in two adjacent cells, produced glows that were diffuse over the entire cell. Then all junctions of the injected cell uncoupled. Presumably the intracellular sequestering capacity was saturated under these conditions. (When mitochondrial sequestering of Ca was blocked by ruthenium red[15], the glow was diffuse even with the shorter injection pulses.) Control injections of 0.15 M KCl did not affect coupling of $[Ca^{2+}]_i$. Massive KCl injections, however, produced transient and diffuse rise of $[Ca^{2+}]_i$ associated with transient uncoupling.

Treatment with ionophore A23187 (Lilly, 2×10^{-6} M) or X537A (Hoffman LaRoche, 1×10^{-5} M) led to enhanced Ca influx which was detectable within 1 min as a diffuse glow that increased progressively during the next 10 min. The rise in $[Ca^{2+}]_i$ was invariably associated with uncoupling, the coupling ratio diminishing progressively with rising $[Ca^{2+}]_i$ (Fig. 3).

Similar results and equally good correlation between rising $[Ca^{2+}]_i$ and uncoupling were obtained when the cells were poisoned with sodium cyanide (5×10^{-3} M) in medium containing Ca as well as in Ca-free medium (Fig. 4).

Prolonged exposure to Ca,Mg-free medium led to a rise in $[Ca^{+2}]_i$. This rise, evidently nurtured by intracellular Ca stores (as Na accumulates inside the cells in Ca-free medium[16], the mitochondria may release Ca^{17}), was asso-

Fig. 3 Ionophore X537A. Exposure to 1×10^{-5} M X537A starts at black arrow and continues throughout the remainder of experiment. Top to bottom: V_I V_{II} ($i = 4 \times 10^{-8}$ A); darkfield television pictures of luminescence (open arrows indicate their time correspondence); coupling ratio V_{II}/V_I; photocurrent P. Cell I depolarises as P rises above background, reaching zero membrane potential at time 9 min. Peak of luminescence in cell II (not shown) occurred 5 min after peak in I. *Top inset:* bright field television picture and cell diagram; cells I and II contain aequorin.
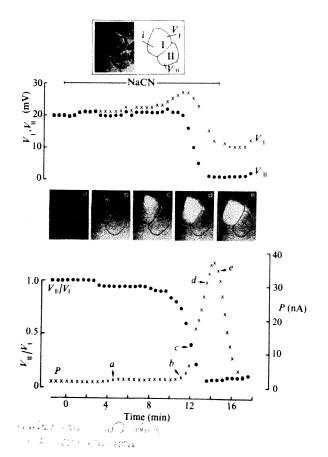
ciated with uncoupling. These experiments were particularly instructive, because $[Ca^{2+}]_i$ and coupling fluctuated: rise and fall in $[Ca^{2+}]_i$ were associated, respectively, with fall and rise in coupling.

In cells with good membrane potentials ($\geqslant 40$ mV), the uncouplings produced by single short Ca pulses generally were spontaneously reversible (Fig. 2). Presumably the cells rid themselves of the excess Ca by sequestering it into mitochondria and by pumping it out. With more massive injections, or after ionophore treatment or prolonged exposure to Ca,Mg-free medium, the uncouplings did not reverse spontaneously. It was then possible to reverse the uncouplings by injection of Ca–EGTA solution yielding $\leqslant 10^{-6}$ M free Ca.

In all these conditions, the rise in $[Ca^{2+}]_i$ was accompanied by depolarisation and fall in input resistance. This effect, a consequence of increased non-junctional membrane permeability to Na^+ (refs 18 and 19), was particularly pronounced when the aequorin glow was diffuse; the depolarisation then paralleled closely the onset of uncoupling. Thus, the question presented itself of whether $[Ca^{2+}]_i$ or depolarisation is the primary cause of uncoupling. To resolve this question, we injected Ca^{2+} while clamping the membrane potential at resting level with a feedback system. Uncoupling ensued none the less whenever the glow reached a junction. Depolarisation is thus clearly not necessary for uncoupling.

In a complementing series of experiments, the cells were exposed to high K (90 mM) medium. Within 2–5 min of application of K medium, the membrane potentials fell to near zero or overshot zero by 5–10 mV. The depolarised cells nevertheless stayed well coupled much longer, in some cases for several hours. Eventually, and rather abruptly, the cells uncoupled; and this was associated with an abrupt rise

Fig. 4   Cyanide. Cells in Ca-free medium. Exposure to 5×10^{-3} M sodium cyanide (top signal). Time correspondence of television pictures *a–e* is marked on the *P* curve. $i = 4 \times 10^{-8}$ A. Cell I depolarises as *P* rises, reaching zero membrane potential at time 17 min.

in $[Ca^{2+}]_i$. Depolarisation is thus also not sufficient for uncoupling.

In conclusion, the cytoplasmic free Ca concentration in the domain of the junction seems to determine the permeability of the junction. We do not know the mechanism by which the calcium ion alters the permeability. Conceivably Ca^{2+} binds to the junctional membrane changing the conformation of the junctional cell-to-cell diffusion channels[2]. We favour this simple notion, because as our results show, the permeability change is readily reversed when the normal $[Ca^{2+}]_i$ is restored in cells with normal Ca-pumping and Ca-buffering ability.

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Modification of a model membrane structure by embedded photochrome

PHOTOREGULATED processes are of interest in diverse biochemical phenomena such as photosynthesis, vision, phototropism and phototaxis. The capture of a photon of appropriate energy, as the first event, triggers, or is followed by, subsequent steps such as electrical potentials or enzyme activity. In this respect, Erlanger and co-workers[1] have shown that some processes such as the catalytic activity of certain enzymes, which are intrinsically insensitive to light, may be modulated or regulated by the presence of appropriate photochromic molecules.

The primary process of photon capture in vision, photosynthesis and perhaps other related phenomena, is the absorption of light energy by 'sensor' molecules Frequently, such 'sensor' molecules form part of, and are embedded in, membranes. The delineation of the step starting from photon absorption by the membrane embedded molecule and ultimately leading to the final response is of considerable importance. Such photoinduced processes may occur by means of a direct link between the 'sensor' molecule and the proteins (or other molecules) connected with the response, with the membrane providing an essentially inert milieu; or they may involve mediation by the membrane in which the 'sensor' molecule is incorporated.

An investigation into the plausibility of the latter proposition would involve monitoring the changes that might occur in the membrane structure on photon absorption by the 'sensor' molecule; in addition, an assay of the activity
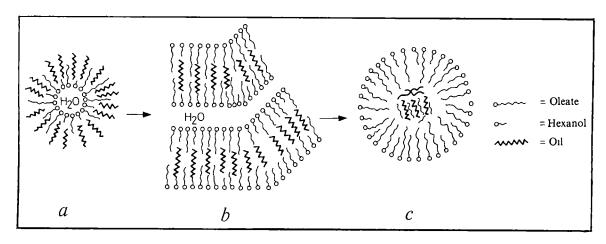
Fig 1 Molecular organisation of the water–oil microemulsions *a c,* clear dispersions of water in oil (0–0 6), and oil in water (water–oil ≥ 1 2), respectively *b*, Birefringent lamellar multi-bilayer system (water–oil = 0 6–1 2)

of a membrane-bound protein (or enzyme) would be desirable Photo-induced changes in the conformation of a model membrane have been observed by Verma *et al*[2], who studied the electron spin resonance of a spin-labelled phospholipid multibilayer film in the presence of incorporated retinal We report here preliminary results on photoinduced conformational changes in a model membrane, and concomitant changes that occur in the esteratic activity of α chymotrypsin, incorporated in the model membrane

Our model membrane system is the microemulsion obtained by the dispersion of water and hexadecane using the amphipathic potassium oleate as the emulsifier and hexanol as the cosurfactant The properties of such systems have been shown by Shah[3] to be relevant to membranes Water–hexadecane (0 7 1 0 to 1 3 1 0 (v/v)) results in a birefringent phase in which the molecular organisation is as shown in Fig 1*b* Compositions with lower ratios yield clear dispersions of water in oil (Fig 1*a*), whereas higher ratios (1 2 1 0 to 1 4 1 0) result in clear dispersions of oil in water (Fig 1*c*) The relevant region is the intermediate water–oil composition depicted in Fig 1*b*, providing a model for membranes as has been verified by several physical measurements[4] We chose this region because it is structurally similar to liposomes, easily monitored by electrical resistance measurements, and, being in the liquid phase, quantitative spectral measurements and enzyme assays can be conveniently done The response of this oil–water lamellar system to drugs and to electrolytes is similar to those of liposomes and of other membrane models[3]

The photosensor molecules chosen were azobenzene and azobenzene-*p*-carboxylic acid methyl ester Both these compounds are known[5] to undergo reversible isomerisation from *trans* to *cis* conformations and back again on illumination with photons of appropriate wavelengths The photoisomerisation produces long lived isomer species which relax with time into the equilibrium isomer population of the predominantly *trans* form In a typical experiment, a small amount of the photochrome was added to the model membrane (photochrome–hexadecane molar ratio 1 300) and the electrical resistance of the membrane followed using the method of Shah[4] Measurements were made on the system over a wide range of water–oil compositions, and in the presence of the photochrome in its equilibrium isomer population, in its *cis* form and the *trans* form, each of which is produced by flooding the membrane containing the photochrome with light of appropriate wavelength for several minutes The variation in the resistance of the microemulsion as a function of the water–oil ratio is shown in Fig 2 We were particularly interested in the region where the system adopts a liquid crystalline lamellar molecular arrangement The

arrow A in Fig 2 shows the resistance changes that occur in the model membrane when the photochrome is in its equilibrium isomer population (on the curve), on converting it completely to the *trans* isomer (illumination with a 250 W mercury lamp using a Corning CS 373 filter 400–500 nm window for 8 min, resistance at lower end of arrow A), and photoisomerising it completely into the *cis* form (using same lamp with a Corning CS 760 filter, 300–400 nm window for 8 min, resistance corresponds here to the top of arrow A) On standing, the photochrome reverts to its equilibrium isomer ratio and the resistance of the system also returns to the original value on the curve Such changes in the resistance are reversible over several cycles of photoisomerisation, and occur both with azobenzene or the azobenzene ester as the photochrome The photochrome molecule, itself non-conducting, does not contribute to the resistance, that is, the variation in resistance with composition shown in Fig 2 is essentially the same with or without the photochrome, in the absence of light Also, there is no change in the resistance of the system on illumination in the absence of the photochrome, thus discounting the possibility of photoinduced perturbation of the microemulsion structure by itself Changes in the resistance of the type shown in arrows A and B of Fig 2 also occur, but less significantly, in the clear regions of the microemulsion

The changes in the resistance may result from a perturbation in the molecular organisation within the lamellar model membrane induced by the conformational change occurring in the incorporated photochrome on photoisomerisation This interpretation is supported by the reversible changes from the birefringent phase to the clear phase and back, observed on the *trans* to *cis* to *trans* interconversion of the photochrome, in the microemulsion of water–oil composition (0 7 1 0) corresponding to the borderline situation between clear dispersion and the lamellar phase Shah[4] has observed anomalous changes in the resistance of such lamellar water–hexadecane system on the introduction of anaesthetic drugs, and has interpreted them as resulting from perturbations in the molecular organisation within the lamellae Our results and interpretation agree with those of Verma *et al*[2] who monitored the electron spin resonance signals of a spin label incorporated in a similar bilayer model system It therefore seems possible to induce perturbations in the structure of a model membrane by altering the geometric shape or conformation of an imbedded photochrome molecule

To investigate whether such membrane structural perturbations could result in a change in the activity of an enzyme incorporated in the membrane, we monitored the ester hydrolytic activity of α chymotrypsin dissolved in the

membrane containing azobenzene and the azobenzene ester separately We chose the lamellar region of the emulsion, and the simplified Bender–Kezdy method of assay[6] with p-nitrophenyl acetate as the substrate The activity of the enzyme was monitored by incubating the solution of the enzyme in the membrane with the substrate in the presence of azobenzene for 10–15 min and spectrophotometrically following the liberated p-nitrophenol absorbance with time (see Fig 2) The photochrome–azobenzene carboxylic ester was unsuitable since it was found to directly bind to chymotrypsin Azobenzene showed no appreciable binding or modification of the catalytic activity of chymotrypsin in an aqueous buffer solution control If the activity of chymotrypsin towards p-nitrophenyl acetate in the model membrane in the presence of the equilibrium population of azobenzene is taken as unity, the activity when the azobenzene was exclusively in the *cis* form (photoisomerised at 300–400 nm for 10 min) was found to be fourfold greater, whereas the activity of the enzyme in the presence of exclusively *trans* photochrome (irradiation at 400–500 nm for 8 min) was about 0 6 Since azobenzene does not seem to bind directly to the protein in our system or modify its catalytic activity in the control, and since chymotrypsin itself is insensitive to light[1], these results have been interpreted as follows Photoisomerisation of the 'sensor' photochrome causes a rearrangement of the membrane structure, and this structural change results in a slight conformational perturbation in, and therefore the activity of, the membrane-bound protein

These model studies may be of relevance to the photophysical changes that occur in the rhodopsin molecule, on bleaching, in the retinal rod outer segment disk membranes On bleaching, the rhodopsin molecule 'sinks' into the membrane[7] It is possible that photoisomerisation of the retinal molecule alters the molecular organisation of the
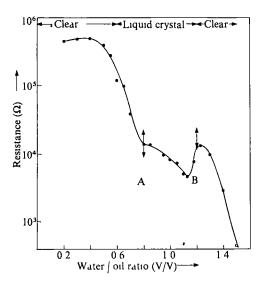
Fig 2 Variation of the electrical resistance of the water–oil dispersions with composition Photochrome (1 5 mg) is added to a mixture containing 1 ml hexadecane, 0 2 g potassium oleate and 0 4 ml hexanol The amount of water is varied to obtain appropriate compositions The resistance was measured using a Philips PR 9500 conductivity bridge at 1 KHz, with platinum electrodes 0 8 cm apart Temperature 28 ± 1 °C For the assay, 0 6 mg of p-nitrophenyl acetate in 0 2 ml hexanol was added to a mixture of 0 38 ml hexanol, 1 0 ml hexadecane, 1 0 ml H₂O, 3 2 mg azobenzene, 0 2 g potassium oleate and 3 mg chymotrypsin (σ) that has been irradiated for 10 min at the chosen λ The A_{400} of the released p-nitrophenol was measured at 90 s intervals and the rate of release was calculated from the linear (later) portion of the release curve The reference cuvette contained the same solution (minus substrate) and was taken through all the steps as the sample Path cells (1 cm) were used in a Beckmann DU instrument (28 ± 1 °C)

disk membrane in such a way as to accommodate the protein deeper into the membrane

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Effect of temperature on immune damage of liposomes prepared in the presence and absence of cholesterol

SINCE the report by Kinsky[1] that liposomes containing lipid haptens could be damaged by activated complement, it has become generally accepted that the lytic attack mechanism of the complement system acts on the lipid matrix of the cell membrane In spite of the accumulation of experimental observations[2] concerned with liposomal damage by activated complement, there is little detailed information about the mechanism by which the damage occurs Inoue and Kinsky[3] suggested that complement did not damage liposomes as a result of degradation by 'phospholipase' (see also ref 4) It was therefore postulated that the lytic action of complement may resemble that of 'detergent'[2–4] Hydrophobic regions in the terminal complement components may penetrate into lipid bilayers, diminishing the non-polar intermolecular attractive forces between lipids Muller-Eberhard et al [5] proposed a model for the molecular assembly of the C5–9 complex on the surface of cells under attack by complement They suggested that complete assembly of the C5–9 complex augmented the detergent effect of C5

We reported previously that liposomal damage induced by lysolecithin[6], polymyxin B (ref 7) and Prymnesin[8] required certain states of fluidity in lipid bilayers, but it is not known whether complement-induced liposomal damage is also influenced by fluidity of bilayers We report here that, using dipalmitoyllecithin liposomes, the complement action requires some fluid conditions of membranes

Immune-sensitive liposomes were prepared using the methods of Kinsky et al [9] and dipalmitoyllecithin liposomes without cholesterol were prepared as described previously[10] Forssman antigen isolated from horse spleen was donated by Dr A Makita, Hokkaido University Antisera against Forssman antigen were prepared by intravenously injecting sheep erythrocyte membranes into rabbits The release of glucose marker was enzymatically assayed as described previously

Dipalmitoyllecithin liposomes (no cholesterol) sensitised with Forssman antigen were incubated with excess amounts of antiserum and guinea pig serum (complement) at various temperatures Damage of the liposomes by antibody and complement was markedly dependent on temperature (Fig 1a) Below 15 °C, the reaction was not detectable, but glucose release was observed at higher temperatures Up to 35 °C, the reaction increased with temperature Since spontaneous release of glucose occurred from the liposomes above 37 °C (ref 10), which resulted from the so-called phase separation of lipid bilayers, the exact temperature dependence of complement-induced permeability change could not be examined above this temperature The
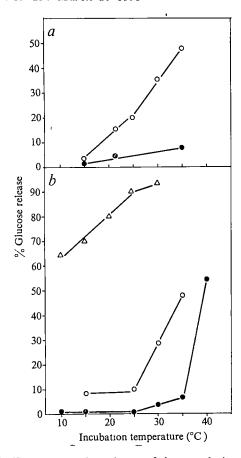
Fig 1 Temperature dependence of liposomal damage by activated complement, methylated bovine serum albumin and Triton X-100 Liposomes were prepared from mixtures of dipalmitoyllecithin and dicetyl phosphate (1 0 1) with or without 10 μg Forssman antigen per μmol phospholipid a, Liposomes without antigen (●) and with antigen (○) were incubated with 11 μl anti-Forssman antiserum and 34 μl guinea-pig serum at various temperatures for 30 min b, Liposomes without antigen were incubated alone (●), with 35 μg Triton X-100 (△) or 12 μg methylated bovine serum albumin (○) for 10 min at various temperatures

by the same procedure The results indicated that sensitivities of liposomes both with and without cholesterol against complement were not significantly different (Fig 3) We therefore concluded that cholesterol does not have any influence on the sensitivity of liposome to antiserum and complement

There was no difference in sensitivity towards antiserum and complement between liposomes of dipalmitoyllecithin, dimyristoyllecithin and egg lecithin, whereas rather different sensitivity was reported between egg lecithin and beef sphingomyelin liposomes[11] Complement-dependent glucose release from dipalmitoyllecithin liposomes, egg lecithin liposomes and dimyristoyllecithin liposomes, all of which contained 50 mol% of cholesterol, was not as influenced by incubation temperature as the release from dipalmitoyllecithin liposomes without cholesterol (Table 1)

Incorporation of cholesterol into dipalmitoyllecithin liposomes has been shown to have complicated effects on the permeability of glucose, it may give a dual effect, that is, the condensing and fluidising effect on bilayers of dipalmitoyllecithin Dipalmitoyllecithin liposomes without cholesterol showed very little reactivity with antiserum and complement at 21–23 °C, whereas those with cholesterol were damaged in the same reaction conditions (Table 1) The effects of cholesterol incorporation on immune damage of liposomes may be caused by the fluidising effect Introduction of cholesterol generally suppressed the sensitivities of liposomes towards various lytic reagents such as lyso-

liposomes without Forssman antigen released little glucose throughout the temperature range 10–35 °C

The effect of antiserum concentration on the loss of marker from dipalmitoyllecithin liposomes which were prepared with a certain amount of Forssman antigen (10 μg per μmol phospholipid) is shown in Fig 2 The experiments were performed at 35 °C From these curves, we determined the amount of antiserum required for glucose release under conditions in which antigen content and concentration of complement source are not rate limiting Liposomes prepared with dipalmitoyllecithin and dicetyl phosphate showed almost the same sensitivity towards antiserum as those made with a mixture of dipalmitoyllecithin, dicetyl phosphate and cholesterol (Fig 2) The amount of fresh guinea pig serum (complement) necessary for marker release was determined

Fig 2 Effects of antiserum concentration on dipalmitoyllecithin liposomes prepared with Forssman antigen Liposomes containing 10 μg per μmol phospholipid were prepared with and without cholesterol as described in the legend to Table 1 Compositions of liposomes were as follows diaplmitoyllecithin (DPL), dicetyl phosphate (DCP) and cholesterol (Chol) in the molar ratio of 1 0 1 1 (●), dipalmitoyllecithin (DPL) and dicetyl phosphate (DCP), (○) 1 0 1 1

Table 1 The specific damage of liposomes containing antigen by antibody and complement

Lecithin	C14 0		C16 0				Egg	
Cholesterol–lecithin (molar ratio)	1 0		1 0		0		1 0	
Incubation temperature (°C)	21–23	35	21–23	35	21–23	35	21–23	35
Serum added			% Trapped glucose released within 30 min					
None	0 25	1 52	0	1 87	1 89	8 03	1 0	7 7
GPS + AS	60 7	68 5	53 3	58 9	16 5	47 2	70 2	77 9
Heated GPS + AS	2 29	1 52	1 40	0 46	1 42	7 56	2 55	6 73
GPS	0	0 38	0	0	0 95	8 50	3 85	3 36

Liposomes were prepared from various lecithins and 10 μg Forssman antigen per μmol phospholipid without or with cholesterol Glucose release was determined after 30 min incubation at 21–23 or 35 °C with various sera as indicated The amounts of each serum added were as follows guinea pig serum (GPS), 35 μl, heated guinea pig serum (heated GPS), 35 μl, rabbit anti-Forssman serum (rabbit AS), 30 μl
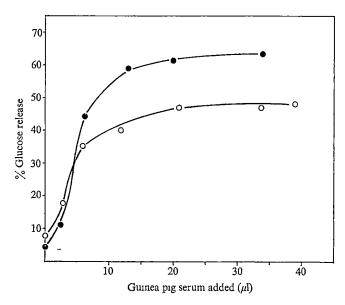
Fig 3 Effects of guinea pig serum concentration on dipalmitoyl-lecithin liposomes prepared with Forssman antigen Liposomes containing 10 μg per μmol phospholipid were prepared with (●) and without (○) cholesterol The liposomal compositions were as described in Fig 2 Glucose release was determined after liposomes were incubated with varying amounts of guinea pig serum in the presence of 11 μl antiserum at 35 °C for 30 min

lecithin[6], polymyxin B (ref 7) and some basic proteins[12] The condensing effect of cholesterol may interfere with the penetration of these molecules into bilayers, but the suppressive effect of cholesterol could not be observed in the case of complement-induced damage of liposomes Dipalmitoyllecithin liposomes with 50 mol% of cholesterol are among the most resistant liposomes yet reported, that is, they resist the action of Triton X-100 (ref 10), lysolecithin[6], polymyxin B (ref 7) and methylated bovine serum albumin (T K and K I , unpublished) Activated 'hydrophobic' complement components may penetrate into such 'highly condensed' membranes

Methylated serum albumin is one of the basic proteins with ability to damage liposomes The mechanism by which the protein damages lipid bilayers may be considered as follows[13] interaction of positively-charged residues of proteins with negatively-charged components of the membranes, change in conformation of the proteins, penetration of exposed 'hydrophobic' regions of proteins into the bilayers The reaction of dipalmitoyllecithin liposomes with methylated bovine albumin also depends markedly on the incubation temperature (Fig 1b) Temperature dependence of methylated albumin-induced liposomal damage was rather similar to that of immune damage of the liposomes On the other hand, the detergent Triton X-100 could affect the liposomes to almost the same degree over the temperature range 10–35 °C Molecules of Triton X-100 can easily penetrate into dipalmitoyllecithin bilayers, whereas protein molecules can not penetrate into bilayers of membranes whose arrangement is too condensed With increased temperature, arrangement of hydrophobic regions of the membrane becomes loose and allows proteins to penetrate It is quite conceivable to assume that the mechanism of membrane damage by activated complement is rather similar to the mechanism of methylated albumin-induced membrane damage The lower transition or pretransition (about 35 °C) was reported[14] on dipalmitoyllecithin The pretransition has been associated[14] with an increase in mobility of the polar head portion of the lipid The increase in mobility of the polar head portion may allow proteins to penetrate into bilayers

The effect of proteins in altering the phase transition characteristics must also be considered Some proteins could

act as modifiers of the lipid phase transitions Addition of cytochrome C has been reported to lower the transition temperature of lipids[15] Our experimental systems, antibody and activated complement components, may also modify the lipid phase transitions It is possible that the effect of activated complement in lowering the transition temperature of dipalmitoyllecithin may be responsible for our results If true, it should be noted that the binding of antibody molecules could not modify the lipid phase transition

Our results suggest that liposome damage induced by complement requires some fluid condition of membrane Cholesterol does not play an important role in the binding of antibody to antigen on the membrane not in the membrane damage by complement components Simple model systems of immune lysis could be established to investigate the mechanism

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Appearance of β globin synthesis in erythroid cells of Ferrara β[0]-thalassaemic patients following blood transfusion

Homozygous β-thalassaemic subjects from the Ferrara region characteristically do not synthesise β globin[1,2] Some of us have presented previously data which suggest that the addition of ribosome-free supernatant isolated from red blood cells of normal adult individuals or from subjects with homozygous haemoglobin S to Ferrara thalassaemic ribosomal material induces β[A] globin synthesis[3], these results suggested that this *de novo* synthesis was not associated with free normal β[A] globin mRNA From these findings it has been concluded that the mutation of the Ferrara β thalassaemia does not reside in the gene for β globin, which is in fact transcribed into normal β globin mRNA, but in a gene coding for a previously unrecognised factor, indispensable for β globin translation Here we report data which suggest that the reticulocytes from these patients synthesise β globin following normal blood transfusion

Table 1 shows the amount of radioactivity incorporated into α, β and γ globin in the reticulocytes of a homozygous β thalassaemic subject from Ferrara, before and 5, 17, and 24 d after receiving the first blood transfusion α and γ globin synthesis varied only slightly, while the incorporation of radioactive leucine into β globin, undetectable before the treatment, became clearly demonstrable after blood transfusion

As a result of the post-transfusional appearance of β globin synthesis, the ratio of α- to non-α-globin synthesis decreased from 2 6 to more normal values The specific activity of purified

Table 1 ^{3}H-leucine incorporation into α, β and γ globin (separated as in refs 7 and 8) by 0 5 ml of packed red blood cells of a homozygous β-thalassaemic subject from Ferrara, before and after blood transfusion

| | Total radioactivity (c p m) | | | Total globin synthesis (c p m) | % of β globin synthesis | $\dfrac{*^3\text{H-}\alpha\text{-}}{^3\text{H-non-}\alpha\text{-globin}}$ |
Before transfusion	α globin	β globin	γ globin			
30 d before	14,763	0	5,529	20,292	0	2 6
1 d before	15,711	0	6,015	21,762	0	2 6
After transfusion						
5 d after	13,905	4,095	5,520	23,520	42 5	1 4
17 d after	13,440	7,091	5,940	26,571	54 7	1 0
24 d after	15,051	5,514	5,793	26,358	48 7	1 3

0 5 ml of washed red blood cells from a homozygous β-thalassaemic subject from Ferrara, obtained before and after the first blood transfusion at the days indicated, were incubated with ^{3}H-leucine for 2 h at 37 °C, as previously reported[1] After incubation the cells were lysed and the stromal material removed by centrifugation Globins prepared from stroma-free haemolysates by acid acetone precipitation[1] were separated into their individual chains by CM cellulose column chromatography[7,8] The chromatographic pattern was continuously monitored with a Gilford recording Spectrophotometer (Model 2400) at 280 nm and fractions of 2 5 ml were collected Radioactivity determinations were on 0 2 ml from each fraction, in Bray's solution[13], with a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3003), efficiency for tritium 49 %

Total incorporation into α, β and γ globin was calculated on the radioactivity pattern, drawn after background subtraction
 *^{3}H-non-α-globin synthesis = ^{3}H-β-globin + ^{3}H-γ-globin

β globin, obtained from isolated haemoglobin A, was maximal approximately 13 d after transfusion (see Table 2)

The characterisation of the radioactive protein appearing in the β globin region was carried out in a β⁰-thalassaemic subject studied 20 d after he had received the first blood transfusion Following a standard red blood cell incubation with ^{3}H-leucine, haemoglobin A was isolated by chromatography on Amberlite CG-50[4] and converted into globins[5] Globins were purified further by chromatography on Sephadex G-200[6] Finally the β globin chains were isolated[7,8]

The isolated β globin chains were digested with trypsin and the resulting peptides separated by column chromatography[9] The baseline radioactivity was measured by determining the number of counts in four regions of the chromatogram, one before βT₃ (B1), one between ammonia and βT₈ (B2), one before βT₆ (B3) and one after βT₁₅ (B4) The measured radioactivity (c p m) in the fractions was B1 = 39, B2 = 99, B3 = 77 and B4 = 50 The fractions of the leucine-containing peptides βT₁, 2, 3, 5, 9 and 14, and of peptide βT₁₃, were separately pooled, lyophylised and purified further (see legend to Table 3)

After purification of the leucine-containing peptides βT₁, 2, 3, 5, 9, and 14 their specific activities were determined (Table 3) Four non-leucine-containing peptides were also studied, three of them (βT₆, 7 and 15) were analysed directly after the first chromatography, while peptide βT₁₃ was purified further The purification of peptides βT₁, 2, 5, 9 and 14 was successful, with a good yield in terms of absorbance and radioactivity, peptides βT₃ and 13, although pure by amino acid analysis, were recovered in amounts too low to give acceptable information

The analysis of the results (Table 3) was limited to peptides βT₁, 2, 5, 6, 7, 9, 14 and 15, which nevertheless represent a sizeable amount of the β globin molecule and are good samples of the entire chain, being located at the beginning, in the middle and at the end of the molecule As the radioactivity is present in the five leucine-containing peptides in amounts proportional to the amino acid content, and practically absent in the peptides without leucine, considering the amount of peptide recovered (see Table 3), it can be concluded that the radioactive material analysed in the β⁰-thalassaemic subject under investigation after blood transfusion was β globin Furthermore, the calculated specific activity of leucine in the original isolated globin was 518 8 c p m µmol⁻¹ (assuming 1 0 absorbance unit = 1 mg of protein), close to that of the leucine in the isolated peptides

Figure 1 shows the percentage of β globin synthesis, relative to total non-α-synthesis, demonstrated in 65 subjects examined at different times after blood transfusion β Globin synthesis was present in all subjects except two, in these patients β-synthesis was not present after the first blood transfusion, but appeared after the second As shown in Fig 1, the maximum

β globin synthesis occurred approximately between the 15th and the 20th day after blood transfusion Over the same period and in the same subjects the ratio of α-globin synthesis to non-α-globin synthesis decreased from the usual pretransfusion values to unity (see Fig 2) In two patients, globin synthesis by intact erythroid cells was determined following injection of plasma from two normal adult individuals, no β globin synthesis was detected in either case

The kinetics of the appearance of β globin synthesis after blood transfusion derived from Table 1 and Fig 1 indicates that haemoglobin A synthesis occurs in the patient's cells and not in the transfused red cells In fact in the latter case β globin synthesis should be maximal immediately after transfusion and rapidly disappear during the days following

The occurrence of β globin synthesis in thalassaemic reticulocytes has been confirmed in four experiments in which, 20 d after blood transfusion, the erythrocytes of the donor (of O group) were separated from the erythroyctes of the patient (of A group) by means of lectin precipitation[11,12] Practically all β globin synthesising capacity was found in association with the non-agglutinated thalassaemic red blood cells In the same four samples the amount of non-radioactive β globin, relative to

Fig 1 Relative synthesis of β globin as determined by CM cellulose column chromatography in 55 homozygous β⁰-thalassaemic subjects from Ferrara, following blood transfusion No β globin synthesis was detected either before or 2–3 months after transfusion Methods as Table 1
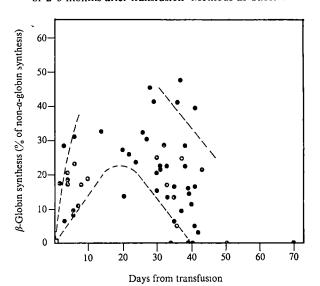
Table 2 Incorporation of radioactivity into purified β-globin by the reticulocytes of a homozygous β-thalassaemic patient from Ferrara, before and after blood transfusion

Days from transfusion	Specific activity
	$(c\,p\,m \times 10^{-3} \times A^{1}_{280\,nm})$
Days before	
5	0
Days after	
5	1 18
10	1 69
17	1 77
24	1 38

1 0 ml of washed packed red blood cells, obtained at the days indicated, were incubated with ^{3}H-leucine for 2 h, at 37 °C Stroma-free lysates, obtained as reported in Table 1, were submitted to Amberlite CG-50 chromatography[4] and the purified haemoglobin A' submitted to CM cellulose column chromatography[7-8] for chain separation The subject is the same as that referred to in Table 1

total non-α-globin, measured as absorbance at 280 nm after CM cellulose column fractionation, ranged from 12% to 21%

Control experiments showed that the above technique effectively discriminated between red blood cells of different groups The lectin agglutination experiments, as well as showing that β globin is synthesised by thalassaemic erythroid cells, exclude the possibility that the appearance of β globin synthesis may be a result of bone marrow graft and proliferation of immature red blood cells, possibly present in the, peripheral blood of the donor

The data reported here strongly suggest the appearance of β globin synthesis in Ferrara thalassaemic erythroid cells after blood transfusion, it is possible that some thalassaemic subjects previously reported in the literature as "β+" may have been transfused β° patients Our findings suggest that the specific inducer of β globin synthesis, previously shown to be present in normal red blood cells[3], can cross the thalassaemic red cell membrane and replace the absent or non-functioning thalassaemic factor in inducing β globin mRNA translation In line with this suggestion is the fact that the maximum of β globin synthesis corresponds approximately with the maximum of red cell destruction Red cell lysis occurs in the reticuloendothelial
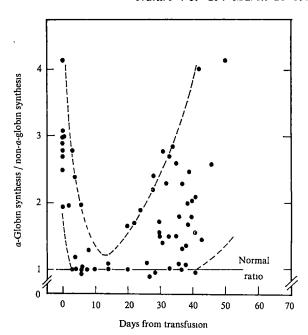
Fig 2 Synthesis of α-globin relative to non-α-globin in 55 homozygous β°-thalassaemic subjects from Ferrara, following blood transfusion

system, which is well represented in the spleen, but also in the bone marrow, in the latter therefore the maturing thalassaemic red blood cells could be exposed to a relatively high concentration of normal red cell cytoplasm and, consequently, of the postulated normal β globin 'inducer'

We cannot distinguish at the moment whether the *in vivo* induced β globin synthesis is directed by transfused or by thalassaemic β globin mRNA So far we have been unable to obtain and transfuse to Ferrara thalassaemic patients blood from subjects homozygous for a mutant stable β globin (as in haemoglobin E or haemoglobin C), which would discriminate between these two possibilities Nevertheless the short survival time of the protein synthesising capacity in reticulocytes together with the presence in Ferrara thalassaemic red blood

Table 3 Incorporation of radioactivity into the tryptic peptides of purified β globin isolated from a homozygous β°-thalassaemic subject 20 d after blood transfusion

Leucine-containing peptides	Molecules of leucine per peptide	Recovery of leucine (μmol)	Recovery of radioactivity (c p m)	Specific activity (c p m per μmol leucine)
βT₁	1	1 305	760	582
βT₂	1	1 271	771	606
βT₃	1	0 059	36	607
βT₅	1	0 325	150	461
βT₉	4	2 588	1,615	624
βT₁₄	1	1 220	823	674
Non-leucine-containing peptides		Recovery of peptide (μmol)		
βT₆	0	2 032	151	—
βT₇	0	2 000	39	—
βT₁₃	0	0 075	43	—
βT₁₅	0	2 109	90	—

The fractions containing the leucine-containing peptides βT₁, 2, 3, 5, 9 and 14, and of peptide βT₁₃, were separately pooled, lyophilised and purified further as follows βT₁, 2 and 14 were purified by column chromatography on Aminex 50W-X2, pyridine-acetate linear gradient from pH 3 1, 0 2 M to pH 5 0, 2 0 M, βT₃ and 13 by two successive treatments on Aminex 50W-X2, pyridine-acetate linear gradient from pH 3 1, 0 2 M to pH 5 0, 2 0 M, and a third column chromatography on Aminex AG1-X2, using the elution system described by Schroeder and Robberson[10], βT₅ by column chromatography on Aminex 50W-X2, pyridine-acetate linear gradient from pH 2 75, 0 1 M to pH 4 25, 1 25 M βT₉ by column chromatography on Aminex 50W-X2, pyridine-acetate linear gradient from pH 3 1, 0 2 M to pH 4 25, 1 25 M The profile of the various peptides after rechromatography was continuously monitored at 570 nm The fractions corresponding to the various peptide peaks were pooled and lyophilised Amino acid analysis was done on 1/40th of the lyophyllised material to check the purity of the peptides after rechromatography, and to determine the leucine content necessary for the specific activity determinations The residual material was used for radioactivity measurement The amino acid analyses proved the purity of the various peptides, the radioactivity blank of the rechromatographies ranged between 15 and 40 c p m

cells of untranslated β globin message[3] are good indications that the post-transfusional β globin synthesis is directed by thalassaemic β globin mRNA In both cases, however, the inducer of β globin synthesis has to become available within the thalassaemic erythroid cells (in which this molecule is lacking or not functioning[3]) to permit β globin synthesis

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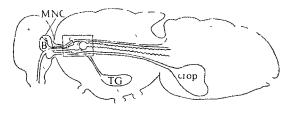
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Neurosecretory cells in insect brain and production of hypoglycaemic hormone

INSECTS have a hyperglycaemic hormone, produced by neurosecretory cells of the corpus cardiacum (CC) This hormone stimulates the production of the major blood carbohydrate trehalose from glycogen stored in fat body cells[1] No hypotrehalosaemic hormone has so far been reported and the level of blood trehalose has been thought to depend on the secretory activity of the CC neurosecretory cells controlled by nerves from the brain[2,3], the relationship between production and consumption of trehalose, which in many insects, including flies, serves as fuel for flight Also, in vitro experiments indicate that medium containing trehalose can inhibit the synthesis or liberation of trehalose from fat body cells, and it has been suggested that such feedback control is an important factor in the natural regulation of trehalose formation[4,5] It was therefore surprising to find that seven decapitated 6-d-old blowflies, Calliphora erythrocephala, not only remained alive for 30 h, during which locomotion and heart beat continued, but that their blood trehalose level had increased from 21 9 g l^{-1} (±4 09 s e m) to 113 2 g l^{-1} (±9 07 s e m)

There are several possible explanations for the extreme hyperglycaemia of the decapitated flies (Fig 1) A hyperglycaemic factor could have been released from the CC, or from the corpus allatum (CA), or from the thoracic ganglion Uncontrolled release might then be ascribed to removal of the brain Alternatively, a hypoglycaemic hormone could be produced in the brain or secreted by some unknown endocrine organ controlled by the central nervous system

Several surgical experiments (Table 1) were performed as previously described[8], except that the use of Ringer was avoided Close proximity of the functional elements (Fig 1) makes it impossible to settle the matter by a single surgical experiment Cardiacectomy involves removal of the aorta wall adjacent to the CC, which contains axon terminals of the medial neurosecretory brain cells (MNC) Extirpation of the MNC, which seems to cause hyperglycaemia, inevitably injures adjacent tissue, and if nerves controlling the CC were affected, the effect might be caused by uncontrolled secretion of CC hormone This possibility, however, is eliminated by the fact that hyperglycaemia also occurred after the CC had been removed A similar effect results from severing of the cardiacrecurrent nerve (CRN) This operation inactivates the CC (refs 2 and 3), but it also prevents the release of brain hormone by interrupting the pathway to the neurohaemal organ (Fig 1) Cutting of the ventral nervous connective is without significant effect, whereas allatectomy results in a slight hyperglycaemia within 20 h
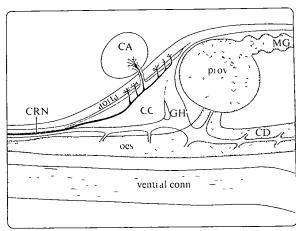
Fig 1 Position of the main elements of the central nervous system and the neuroendocrine system in the blowfly, together with the anterior part of the aorta and of the alimentary canal with crop and crop duct The retrocerebral endocrine complex (inset) illustrated as a simplified diagram below B, Brain, TG, thoracic ganglion, CRN, cardiac-recurrent nerve, CA, corpus allatum, CC, corpus cardiacum, GH, hypocerebral ganglion, oes, oesophagus, MG, midgut, prov, proventriculus, CD, crop duct, MNC, medial neurosecretory brain cells The main axis of the central nervous system, brain–ventral connective–thoracic ganglion, is severed by decapitation, as is the main axis of the neuroendocrine system, brain neurosecretory cells–CC–aorta wall–CA, since the CRN is severed This compound nerve contains both neurosecretory and non-neurosecretory axons, the latter belonging to the stomatogastric nervous system Axons from the MNC of the brain (aldehyde fuchsin positive) penetrate the CC (ref 6) to terminate in the aorta wall dorsal to the CC This region is their neurohaemal organ Some MNC axons, however, enter the CA in adults[7]

Table 1 indicates that the extreme hyperglycaemia is unlikely to be caused by any hyperglycaemic factor, from the ventral nervous axis or from the neuroendocrine axis Rather, hyperglycaemia occurs after elimination of secretion, either of a brain hormone or CC hormone or both, and so seems to be caused by the lack of a depressing factor The CC produces a hyperglycaemic hormone[1-3,5,9] The other possibility, that a brain hormone, transported to its neurohaemal organ near the CC by way of the CRN, has a hypoglycaemic function,

Table 1 Haemolymph trehalose level given as g l⁻¹ ± s e m before and after various surgical experiments on adult male and female blowflies

	Trehalose average $(g \, l^{-1})$	n	Significance t test
Female blood, before operation	27 5 ±0 92	45	
Male blood, before operation	26 4 ±1 22	60	
Pooled female and male before operation	26 8 ±0 80	105	
Pooled female and male before operation*	26 1 ±1 40	9	
MNC extirpated, 20 h after operation	63 6 ±3 67	8	$P < 0\,001$
Cardiacectomised, 20 h after operation	49 9 ±4 07	9	$P < 0\,001$
CRN severed, 20 h after operation	53 7 ±3 24	29	$P < 0\,001$
CRN severed, 24 h after operation*	56 9 ±7 60	9	$P < 0\,001$*
Allatectomised, 20 h after operation	36 6 ±1 96	42	$P < 0\,001$
Ventral nerve cut, 20 h after operation	22 7 ±2 83	8	$P \sim 0\,1$

Samples from operated flies fed sugar and water only compared with pooled ($n = 105$) samples taken from females and males before operation (then 6 d old)

* Flies (3d old at operation) fed both meat, sugar and water before and during the 24 h before second blood sampling They were treated separately for t test (difference between first and second blood samples)

seems likely, also in view of the ability of MNC extracts to revert the hyperglycaemia (Table 2)

The moderate hyperglycaemia produced by allatectomy may fit with observations indicating that the CA positively regulates the MNC (refs 10 and 11) When allatectomised adults were treated at the time of operation with *Cecropia* juvenile hormone (JH), applied topically on the abdominal cuticle, a trehalose concentration of only 7 5 g l⁻¹ (±2 71 s e m, $n = 9$) was found 20 h later When JH had been applied 10 h after the operation, a trehalose level of 16 3 g l⁻¹ (±1 58 s e m, $n = 8$) was observed after another 10 h Application of JH to ten non-allatectomised flies did not, however, affect blood trehalose concentration

ever, normally derives from ingested sugar (sucrose) rather than from net fat body glycogen conversion to trehalose Nine flies were given water but not sugar from emergence, and 12 h later the CRN was severed At that time the blood contained 17 7 g l⁻¹ trehalose (±0 72 s e m) Blood samples taken 24 h after the operation, during which period they were still given water only, contained 13 0 g l⁻¹ trehalose (±1 10 s e m) They were then given sugar *ad libitum* and in 24 h the blood trehalose level then increased to 44 3 g l⁻¹ (±2 70 s e m)

In mosquitoes the MNC have been shown to regulate glycogen and fat synthesis[14,15] In *Calliphora* deprived of their MNC, the fat body cells contain much glycogen but only little fat[8], and the same seems to hold for fat body cells after

Table 2 Effect of brain extracts (with and without MNC) on flies rendered hyperglycaemic by previous severing of the cardiac-recurrent nerve

	Trehalose average $(g \, l^{-1})$	n	Significance t test
CRN severed, 22 h after operation	52 3 ±3 21	16	
2–3 h after injection of MNC extract	27 8 ±9 66	8	$P < 0\,02$
2–3 h after injection of brain extract	51 2 ±7 02	7	

Each of 16 hyperglycaemic recipients was injected with a crude, freshly prepared extract (homogenate) in Ringer (made by means of a small Potter–Elvehjem type homogeniser) of either three groups of MNC from 7-d-old donors ($n = 8$) or of a similar amount of brain tissue from the same donors, not containing MNC ($n = 7$, 1 died)

In adults the neuroendocrine system is geared to make a relatively high concentration of blood trehalose available at the onset of flight This may partly be caused by a low activity of the MNC In larvae, in which high activity of the MNC and of the CA is linked with developmental events, the trehalose level would consequently be expected to be lower Indeed, in Dipteran larvae including *Calliphora*[12], trehalose has been reported to be very low or absent[13] Preliminary analyses of trehalose concentrations showed 0 5–3 5 g l⁻¹ ($n = 6$) in second instar larvae, 2 8–10 2 g l⁻¹ in third instar larvae ($n = 9$), and up to 16 g l⁻¹ in pupae ($n = 5$) Haemolymph trehalose was also determined in different developmental stages of the butterfly *Pieris brassicae* (Table 3) Again, the more active the MNC and the CA are known to be, the smaller is the content of trehalose in the blood

Hyperglycaemia could be induced both in sugar-fed flies and meat-fed flies (Table 1) Increased blood trehalose, how-

severing the CRN Thus, lipid catabolism may be accelerated as in diabetic vertebrates

Hyperglycaemia was associated with blood hyperosmolarity ranging from 600–1200 mosmol (normal osmolarity 350–400 mosmol) This may be the cause of the polydipsia which developed on removal of the MNC or cutting the CRN within 10–12 h the flies increased in weight from the normal 65–75 mg up to 130 mg Extreme bloating resulted not only from distension of the crop, but also be increased haemolymph volume Such polydipsia in blowflies has been described[8,16], but bursting at weak points of the cuticle often occurs during the night, and so may have passed unnoticed In fact much blood is lost and by continued polydipsia haemolymph composition must be greatly disturbed This, together with the hypertonicity, is bound to have harmful effects on most tissues Since several abnormalities, which seem to result from lack of brain hormone, at least superficially resemble diabetic conditions in vertebrates, it was tempting to test whether insulin had any effect in blowflies (Table 4) No interpretation seems warranted until more knowledge of the endocrine system in blowflies has been obtained and it should be added that no significant effects of injection of insulin into normal intact flies has so far been found

In conclusion, it is suggested that MNC have an important role in the regulation of carbohydrate metabolism When they are removed or prevented from secretion, hyperglycaemia ensues, accompanied by blood hypertonicity, polydipsia, lipid catalysis and fatigue (decreased and slow locomotor activity, perhaps resulting from impeded uptake of trehalose by the muscles) These effects should be related to other findings on

Table 3 Haemolymph trehalose (g l⁻¹ ± s e m) at different developmental stages of the butterfly *Pieris brassicae*

	Trehalose average $(g \, l^{-1})$	n
Third instar larvae	12 5 ±1 39	7
Fifth instar larvae	16 9 ±0 49	7
Non-diapausing pupae	14 5 ±2 17	8
Diapausing pupae	32 4 ±1 13	9
Adults	29 7 ±4 17	9

Diapausing pupae (MNC resting) contain significantly ($P < 0\,001$) more trehalose in the blood than non-diapausing pupae, in which the MNC are active

Table 4 Effect of injecting 500–800 IU kg⁻¹ crystalline ox insulin (insulin) or zinc protamin insulin (ZPI) into hyperglycaemic flies (CRN previously severed)

	Trehalose average (g l⁻¹)	n alive	n died
CRN severed 24 h earlier	53 5 ±3 65	20	
18 h after injection of insulin	23 6 ±5 28	7	1
18 h after injection of Ringer	57 1 ±5 45	8	4
3 h after injection of insulin	41 5 ±4 37	5	
15 h after injection of ZPI	37 8 ±5 87	7	
3 h after second dose of ZPI	29 6 ±4 28	7	

Calliphora-Ringer (1 µl) containing insulin, or Ringer alone (1 µl) was injected after the removal of superfluous blood For about a minute after the insulin injection there were convulsions of the somatic musculature, and increased locomotor activity continued for up to 20 min Otherwise, insulin and ZPI were well tolerated, and the mortality on the second day after severing the CRN was generally lower than in non-treated flies (of which 20–30% died on the second day) The trehalose level before injection was only determined for the first 20 flies (the first experiment and control), since the average value remained close to that in Table 1 (CRN severed)

MNC function and unless MNC hormone(s) has a diversity of functions, it should be determined which effects are secondary to which, when results of MNC extirpation are considered

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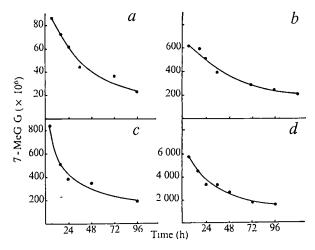
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tibility to these carcinogens We report here results which may provide an explanation for this difference and also for the observation that large doses of dimethylnitrosamine are carcinogenic to the kidney while small doses are not

The major product of the reaction with DNA of metabolites derived from nitrosamines is 7-alkylguanine but other sites are also attacked—alkylation of the O^6 and 3 positions of guanine, the 3 position of cytosine, the 3 and 7 positions of adenine and the O^4 position of thymine have been detected[6-8] O^6-Alkylguanine, in particular, has been shown to miscode and is therefore potentially mutagenic[9,10]

Fig 1 Formation and subsequent loss of 7-methylguanine (7-MeG) from rat kidneys (*a* and *c*) and liver DNA (*b* and *d*) after administration of dimethylnitrosamine Female Wistar rats (160 g) were treated with either 2 5 mg per kg body weight (*a* and *b*) or 20 mg per kg body weight (*c* and *d*) of ¹⁴C-dimethylnitrosamine (DMN) (specific activity 28 mCi mmol⁻¹ and 4 mCi mmol⁻¹, respectively, prepared as previously described[4]) At various times after the injection, DNA was isolated from kidney and liver[4], hydrolysed in 0 1 N HCl (refs 7 and 11) and the liberated purines separated by column chromatography on Sephadex G10 or Dowex-50 (refs 7, 8 and 11) The amounts of 7-methylguanine and O^6-methylguanine present were determined from the radioactivity in the fractions corresponding to the positions of authentic unlabelled markers, the amount of guanine present was determined from the absorbance of the guanine fractions The results are expressed as the proportion of guanine methylated at the 7-position (7-MeG) to total guanine (G) present

Effect of dimethylnitrosamine on persistence of methylated guanines in rat liver and kidney DNA

DIMETHYLNITROSAMINE IS a potent carcinogen in many species, inducing tumours of the liver, kidney and lung[1,2] It is now well established that the carcinogenic activity of this compound is caused by its metabolic transformation within susceptible tissues to a chemically reactive agent which methylates a variety of cellular macromolecules[1-3] In spite of the fact that metabolism of dimethylnitrosamine is eight times greater in the liver than in the kidney, thus the alkylation of macromolecules is eight times greater in liver than kidney, a single large dose of dimethylnitrosamine given to the adult rat induces tumours of the kidney but not of the liver[1,4] (Liver tumours are produced in the adult rat only if dimethylnitrosamine is given after partial hepatectomy[5] or by repeated small doses or prolonged feeding[1,2]) Similar experiments with certain nitrosamides have shown that although they penetrate and subsequently react to alkylate cellular components to a similar extent in many tissues, they do not produce an equal incidence of tumours in all these organs[1-4] It seems therefore, that different tissues vary quantitatively in their inherent suscep-

Further, when a range of alkylating carcinogens of different potency are compared, the ability to form this product correlates much better with the carcinogenicity than the ability to form 7-alkyldeoxyguanosine or the other minor products described above[3,7,11] One possible explanation for the greater sensitivity of the kidney than the liver to a large single dose of dimethylnitrosamine would be any difference in the ability of the tissues to repair lesions produced in DNA before cell division We have therefore studied the persistence of O^6-methylguanine and 7-methylguanine in DNA of rat liver and kidney after a large dose (20 mg per kg body weight) of dimethylnitrosamine which would produce some kidney tumours and a small dose (2 5 mg kg⁻¹) which would not and have found a marked difference in the rate of removal of O^6-methylguanine from the kidney DNA These results may have considerable importance in determining the tissue specificity of tumour production by nitroso compounds and provide further support for the recent hypothesis[12] that the rate of elimination of O^6-alkylguanine from DNA may be an important factor in neoplastic transformation by alkylating carcinogens

After a dose of 20 mg kg⁻¹ dimethylnitrosamine, the 7-methylguanine formed in liver and kidney DNA was lost at a rate corresponding to a half life of about 60 h (Fig 1) A similar rate of loss was found in kidneys of rats treated
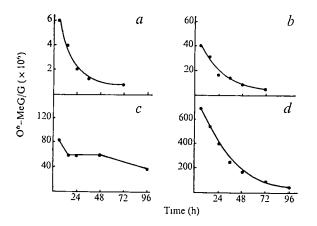
Fig. 2 Formation and subsequent loss of O⁶-methylguanine in rat liver and kidney DNA after administration of dimethylnitrosamine Proportion of guanine methylated at the O⁶ position (O⁶-MeG) to total guanine (G) present in DNA Experimental procedure as in Fig 1

with 2 5 mg kg⁻¹ but the rate of loss in the liver was slightly slower after this dose These data are in reasonable agreement with those published[8,13-15] for various doses and shows that 7-methylguanine is lost from DNA *in vivo* at a rate somewhat faster than that expected from chemical depurination at neutral pH (ref 16) The possible contribution to this loss from the necrosis of highly alkylated cells and dilution by new DNA synthesis could not be determined accurately, however, and it is therefore not possible to determine to what extent an enzymic excision mechanism contributes to the removal

Similar data for the formation and loss of O⁶-methylguanine are shown in Fig 2 In both tissues shortly after administration of the carcinogen the amount of O⁶-methylguanine formed was only about 10% of the amount of 7-methylguanine O⁶-methylguanine in DNA is chemically stable at neutral pH (refs 8 and 17) but this product was lost from the DNA of liver after either dose with a half life of about 20 h This is much faster than the loss of 7-methylguanine and therefore could not have resulted from cell death or DNA replication, which would affect both products equally Clearly, an enzyme system not yet characterised is responsible for the removal A similar system must also be present in the kidney, since O⁶-methylguanine was lost from the kidney DNA after the low dose of dimethylnitrosamine at a rate only slightly slower than in the liver After the larger dose, however, there was a striking difference between the two tissues in the persistence of O⁶-methylguanine in DNA In the liver (where neither the large nor the small dose is effective in inducing tumours) O⁶-methylguanine was lost from DNA at a similar rate after both doses, but in the kidney after the large dose (which does induce kidney tumours) there was an initial fall of about 30% in the amount of O⁶-methylguanine present in DNA between 6 and 15 h after giving the nitrosamine, but there was practically no decline for 50 h and only a slow rate of loss after this period (Fig 2)

Thus the potentially mutagenic O⁶-methylguanine is much longer-lived in kidney DNA after a large dose of dimethylnitrosamine than after a smaller dose or in liver DNA after either dose This correlation with the induction of tumours, together with recent data showing that the brain, which is much more sensitive than the liver to the carcinogenic activity of nitrosamides, is much less active than the liver in catalysing the removal of O⁶-alkylguanine from DNA (refs 12 and 18), provide support for the hypothesis that the differing susceptibilities of organs towards carcinogenic stimuli may be determined by the ability to repair certain alterations produced by the carcinogen in DNA This

ability seems to vary both from tissue to tissue and with the dose of carcinogen used It is not yet known why the loss of O⁶-methylguanine from kidney DNA is so much slower after the larger dose of dimethylnitrosamine, but it is possible that the enzyme system responsible for the removal is somehow inhibited by the large dose of the carcinogen or alternatively, has only a limited capacity to remove this product Further experiments to study these possibilities and characterise the enzymic reactions involved are in progress This process may involve a similar activity to that recently demonstrated in microorganisms[17,19]

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Base changes in the recognition site for *ter* functions in lambdoid phage DNA

THE DNA of bacteriophage λ is a linear duplex of about 45,000 base pairs with single-stranded projections of twelve bases at its 5′ termini[1,2] These projections are of complementary base sequence[3] and result from the action of a λ gene product[4], *ter*, on a concatenated form of the phage DNA[5] The base sequences of the single-stranded regions have been determined from repair reactions with DNA polymerase and radioactively labelled nucleoside triphosphates[3] and from analyses of DNase digestion products from 5′-terminally labelled DNA[6] The 3′ terminal sequences of λ DNA have also been determined by analysis of DNase digests of DNA labelled at or near its 3′ termini in exonuclease-repair reactions with T4 DNA polymerase[7] Together, these results gave a sequence of 25 base pairs in the region of the DNA molecule at which the *ter* function interacts (*cos*) The sequence between the nicks that the *ter* enzyme would be required to make in the concatenated DNA is bisected by an axis of twofold rotational symmetry This sequence provided an example of discontinuous or hyphenated symmetry in a DNA sequence recognised by a protein[7] The base sequences of the *lac* operator[8], the λ promoter[9], and the promoter[10] for tRNA$^{Tyr}_{suIII}$ also contain hyphenated elements of symmetry

Of the sixteen base pairs confined between the limits of the symmetrically disposed base pairs, twelve are symmetrically arranged about the axis The classical biochemical genetic approach would define the bases essential for recognition by the *ter* system as those that cannot be changed without leading to

loss of function. Mutations of essential bases in the recognition sequence would thus be effectively lethal, whereas mutations of inessential bases could not be detected readily. Comparison of sequences from different lambdoid phages whose *ter* systems could cross react might well provide information equivalent to that from a series of mutants. We therefore analysed the 5′ terminal sequences of DNA from several other lambdoid phages[6], some of which had *ter* systems that were known to cross react[11]. The DNA from four of these (φ80, 82, 424 and 21) had terminal sequences identical to those of λ DNA, but we report here the 5′ terminal sequences of DNA from another lambdoid phage[12], φD326, which is closely related to φ80. The sequences differ in two nucleotides from the equivalent sequences in λ DNA.

The 5′ terminal sequences of φD326 DNA were determined by the method used previously for several phage DNA preparations. DNA was extracted from the purified phage by treatment with phenol, dephosphorylated with bacterial alkaline phosphatase, and labelled at its 5′ termini with ^{32}P using the polynucloetide kinase reaction. The labelled DNA was purified and digested with pancreatic DNase and the oligonucleotides fractionated by ionophoresis on AE81 and DE81 ion-exchange papers[13], and by two-dimensional ionophoresis on cellulose acetate and thin layer chromatography on PEI cellulose[14] (Fig. 1). The base sequence of each radioactive oligonucleotide was determined from ionophoretic analysis of its products on partial digestion with venom phosphodiesterase. The sequences deduced (Table 1) for the cohesive ends of φD326 DNA are
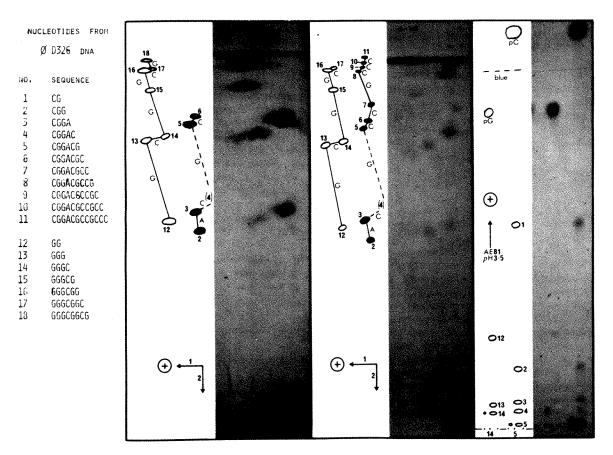
Fig. 1 Nucleotide maps of DNase digests of terminally labelled DNA from φD326. Stocks of the phage were prepared as described previously[12] and purified by two cycles of equilibrium centrifugation in CsCl (41.5% w/w). DNA was isolated from the dialysed phage preparation by rolling gently with freshly distilled phenol (three times) and was dialysed against four changes of 0.01 M Tris-HCl, *p*H 8.0, 1 mM EDTA. Terminal phosphate groups were removed with bacterial alkaline phosphatase (Whatman, UK; 25 μg ml⁻¹ (2 h at 37 °C)) which was then eliminated by further extraction with phenol and the preparation dialysed against 0.02 M tris-HCl, *p*H 7.5. Labelling with polynucleotide kinase from *Escherichia coli* B infected with phage T4 and γ-^{32}P-ATP (specific activity, 14.8 Ci mmol⁻¹; Radiochemical Centre, Amersham, UK), and purification of the labelled DNA by sedimentation through 6–20% w/v sucrose gradients were carried out as described previously[6,13]. The DNA (170 μg, 73,000 c.p.m, Cerenkov counts [16]) was divided into five portions for digestion with pancreatic DNase in 0.01 M Tris-HCl, *p*H 7.5, 5 mM MgCl₂. Two portions were digested at DNase concentrations of 25 μg ml⁻¹ (37 °C, 3 h) and the products fractionated by ionophoresis on AE81 paper at *p*H 3.5 or DE81 paper at *p*H 2.0 to give oligonucleotides 1–5 and 12–16 the sequences of which were determined from analyses of partial digests with snake venom phosphodiesterase (Table 1). The remaining three portions of the terminally labelled DNA were digested with the same enzyme under milder conditions (2–10 μg DNase ml⁻¹; 60–90 min at 23 °C) and fractionated by ionophoresis on cellulose acetate at *p*H 3.5 (in 7 M urea) followed by transfer[17] to thin layers of PEI-cellulose (Macherey Nagel AG, Germany) for development in 1.6 M formic acid adjusted to *p*H 3.6 with pyridine[14]. The oligonucleotides were eluted with 30% v/v triethylamine carbonate and allotted to their respective family on the basis of the 5′ terminal mononucleotide identified by ionophoresis on AE81 paper, *p*H 3.5, after complete digestion with venom phosphodiesterase[13] (0.1 mg ml⁻¹ in 0.02 M Tris-HCl, *p*H 8.5, 37 °C, 1 h). In the drawings accompanying the photographs, oligonucleotides with a 5′ G terminus are shown as open rings and those with a 5′ C terminus as filled rings. The sequence of oligonucleotides 2–6 and 12–16 was inferred from their behaviour on the two-dimensional system and confirmed by ionophoretic analysis of partial digests with venom phosphodiesterase.(Table 1) on AE81 paper at *p*H 3.5; examples of these on oligonucleotides 5 and 14 are included on the right of the figure. (Oligonucleotide 4 was obtained from the DNase digests fractionated by ionophoresis on AE81 and DE81 papers and was found in partial digests of oligonucleotides 5 to 9 with venom phosphodiesterase, but it was not found in the milder DNase digests analysed two-dimensionally. Its position on the two-dimensional nucleotide maps, shown in parentheses, has therefore been estimated from the difference in base sequence of nucleotides 3 and 5 and the effects on ionophoretic and chromatographic behaviour resulting from addition of C and G residues to oligonucleotides generally[18,19].) The sequence of the longer oligonucleotides was deduced from their behaviour on the two-dimensional separation system, as changes in position are diagnostic of the nucleotide by which two immediate homologues differ[6,18,19] (these are indicated alongside the lines joining the oligonucleotides from the two 5′ terminal families in the drawings to the left of the photographs) and from the necessary complementarity of the two terminal sequences. The different extents of digestion of the two termini of the DNA in the samples shown above is probably fortuitous.

Table 1 Analysis of nucleotides separated by two-dimensional ionophoresis and thin-layer chromatography

Nucleotide no.	Mobility AE	DE	5' Terminus	Products from partial digestion with venom phosphodiesterase				Base sequence of oligonucleotide
1	0.57		C					CG
2	0.16	0.80	C	CG,				CGG
3	0.070	0.57	C	CG,	CGG			CGGA
4	0.044		C	CG,	CGG,	CGGA		CGGAC
5	0.012		C	CG,	CGG,	CGGA,	CGGAC	CGGACG
6	0.007		C		CGG,	CGGA,	CGGAC, CGGACG	CGGACGC
12	0.26	0.82	G					GG
13	0.066	0.26	G	GG				GGG
14	0.045	0.25	G	GG,	GGG			GGGC
15	0.012		G	GG,	GGG,	GGGC		GGGCG
16	0.003		G	GG,	GGG,	GGGC, GGGCG		GGGCGG

Oligonucleotides have been grouped to show the families of progressively overlapping sequences from each 5' terminus of the DNA. Their number refers to their identity in Fig. 1. Mobilities on AE81 paper (*p*H 3.5) and on DE81 paper (*p*H 2.0) are expressed as ratios of the distance moved by the oligonucleotide to that of the blue dye, xylene cyanol FF, and in most cases are the average from several independent experiments. The sequence of each oligonucleotide was deduced from its mobilities and the M values[13,15] of the products of partial digestion with snake venom phosphodiesterase, when each oligonucleotide gave the successively smaller members of its family (Fig. 1). Partial digests of the oligonucleotides (0.01 mg ml[-1] venom phosphodiesterase in 0.02 M Tris-HCl, *p*H 8.5, 20 μl; 23 °C, 20 min and 60 min) were analysed by ionophoresis on AE81 paper at *p*H 3.5 or DE81 paper at *p*H 2.0 (ref. 13).

Fig. 2 The 5' terminal sequences of φD326 DNA. The longest oligonucleotide sequences deduced (Fig. 1) have been base-paired and extended by addition of the complementary bases to show the double-stranded structure that would be formed by cohesion of the two single-stranded ends.

written as a base-paired structure in Fig. 2. This structure, like that of the cohesive ends of λ DNA possesses a twofold rotational symmetry; the symmetrically disposed bases are shown enclosed in boxes. The two bases in which this sequence differs from the corresponding region of λ DNA are underlined and both changes are in non-symmetrical positions.

The difference between terminal nucleotide sequences of φD326 DNA and λ DNA could reflect a different sequence requirement for the φD326 *ter* system, or it could indicate that the bases in these positions are unimportant for the *ter* interaction. If the φD326 *ter* system can recognise the appro-

priate nucleotide sequence in λ DNA it should excise λ from a tandem dilysogen. To test this, we followed the genetic approach by which Mousset and Thomas demonstrated the existence of the λ *ter* system.

*Rec*A strains lysogenic for λ*imm*^λ*A*^− and λ*imm*^434^*R*^− (strains *a* and *b* in Fig. 3) were constructed. On the prophage map the *ter* recognition sequence, *cos*, is between the λ genes *R* and *A* and the order of the prophages is defined by the finding that superinfection with the heteroimmune phage λ*imm*^21^ excises λ*A*^−*imm*^434^*R*^− phages from strain (*a*) and λ*A*^+*imm*^λ*R*^+ phages from strain (*b*) (ref. 4). Since the λ site-specific recombination system, *int*, can excise phage λ from a λ dilysogen[4], the heteroimmune superinfecting phage chosen was an integration defective phage (λ*biol*). Phages φ80 (ref. 23) and φD326 (ref. 12) do not integrate at the λ attachment site and therefore have *int* systems with different specificities from that of λ. Phages φ80 and φD326 have the same *int* specificity (N.E.M., unpublished) and since φ80 *int*^+ and *int*^− phages were found to be

Table 2 Heteroimmune superinfection of the λ*imm*^λ−*imm*^434 dilysogens
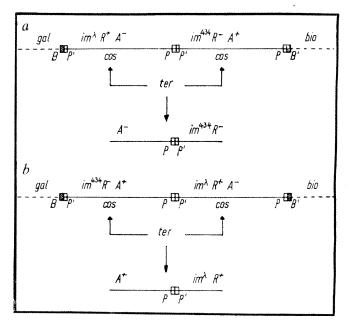
Superinfecting phage	Relative yields of phage with immunity of either prophage	
	Strain (*a*)	Strain (*b*)
λ*imm*^21*biol*	100*	100†
φ80	46*	61†
φD326	45*	99†
—	1	1

* Over 90% of the phages were λ*imm*^434*R*^−*A*^−
† Over 90% of the phages were λ*imm*^λ*R*^+*A*^+
The results are the average of two experiments. The dilysogenic strains were grown at 32 °C and superinfected with heteroimmune phage at a multiplicity of between 3 and 5, as described by Thomas[24]. Following superinfection with λ*imm*^21 the lysates were assayed on the *sup*II host W1485 (λ*imm*^21*Aam*32*Ram*54*am*60) and following superinfection with either φ80 or φD326 on W1485 (φ80). φ80 and φD326 are homoimmune[12]. The plates were incubated at 38 °C so that the λc1857 plaques (that is λ*imm*^λ) were clear and the λ*imm*^434 plaques turbid. Plaques were purified on the *tonA sup*II strain and tested on a *tonA sup*⁰; *tonA* strains are resistant to φ80 (ref. 23) and φD326 (ref. 12). Media and methods were as described previously (ref. 25). φD326 plaques are always variable in size, presumably because this phage adsorbs inefficiently to its host. Since λ*imm*^434*R*^−*A*^− plaques are extremely small, those excised and therefore packaged by φD326 are very difficult to estimate. This probably explains the relative yield of 45% from strain (*a*) as opposed to 99% from strain (*b*).

Fig. 3 Structures of the dilysogenic strains used. The bacterial host was the *recAsup*II strain QR48 lysogenised by λ*imm*^λc1857*Aam*32 and λ*imm*^434*Ram*54*am*60. B.P′, P.P′ and B′.P are the components of the attachment regions[21]. The markers *A* and *R* are closely linked in the prophage map but are at opposite ends of the vegetative map[22]. The *ter* function acts at *cos* and breaks the linkage between *A* and *R*.

equally efficient in excising phage from λ dilysogens, we felt justified in using the efficiencies with which the *int⁻* phage λ *bio1imm*[21] and the *int⁺* phages φ80 and φD326 excise phage λ from the two dilysogenic strains as an index of *ter* activity.

The results (Table 2) show clearly that phages λ, φ80 and φD326 can all supply a function able to excise linear chromosomes terminated by genes *A* and *R* from a structure in which these termini are linked. We conclude that the *ter* function of φD326, like that of φ80 (ref. 11), is able to act not only on its own *cos* sites but on those of phage λ, even though in this case the *cos* sequences differ in two base pairs from those of φD326.

Since the φD326 *ter* system is able to recognise the appropriate nucleotide sequence in λ DNA as well as in its own, the nucleotides in which the terminal sequences of these two DNAs differ cannot be essential for physiological function. Both nucleotide changes occur at positions that are not included in the symmetrically disposed locations. The observed results are therefore consistent with the hypothesis that the symmetrically arranged bases constitute the recognition element of the target sequence for the *ter* system, but they do not, of course, prove this.

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Plasmid-determined antibiotic synthesis and resistance in *Streptomyces coelicolor*

IN eubacteria, many characteristics[1-3] including resistance to antibiotics and other agents are plasmid-determined. In streptomycetes there are indications that plasmid genes are involved in the production of certain antibiotics: kasugamycin and aureothricin by *Streptomyces kasugaensis*[4], oxytetracycline by *S. rimosus*[5] and turimycin by *S. hygroscopicus*[6]. An increased proportion of antibiotic non-

producing strains is found after treatment of antibiotic producers with agents known to cause plasmid loss in eubacteria; in *S. rimosus,* crosses provided additional evidence that extrachromosomal genes are involved in antibiotic production. In all three species, the cause of antibiotic non-production, for example whether it involved an interruption of the biosynthetic pathway or a defect in the liberation of antibiotic into the medium, was unknown. We report here that both the production of and resistance to the same antibiotic in *S. coelicolor* A3(2) are determined by genes borne on the plasmid SCP1.

In *S. coelicolor* A3(2) recombination has been used to map more than 100 genes, identified by auxotrophic, resistance and morphological mutations, to a single circular linkage group[7]. Recombination requires cellular contact and leads to the inheritance from each parent of large chromosomal regions; it thus has at least some of the characteristics of conjugation in the Enterobacteriaceae. Moreover, at least part of the exchange of chromosomal markers between mating strains is determined by a plasmid, SCP1. Genetic evidence for the existence of SCP1 is very strong: first,
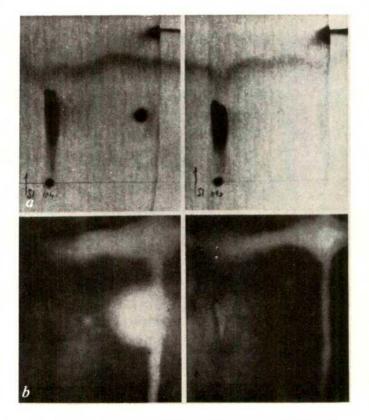
Fig. 1*a*, Two-dimensional chromatography on silica gel thin-layer plates (Eastman Chromagram No. 13181) impregnated with fluorescent indicator of broth concentrates from cultures of (left) SCP1⁺ strain 104 of *S. coelicolor* A3(2) and (right) SCP⁻ strain 1190. Solvent 1 (run bottom to top in the photograph) was tertiary butyl alcohol–acetic acid–water (72:3:25); Solvent 2 (run left to right in the photograph) was ethyl acetate–acetic acid–water (88:6:6). The chromatograms were photographed in 254 nm ultraviolet light. Note the spot of ultraviolet-absorbing material with R_f 0.45 in Solvent 1 (purer material normally runs at 0.75–0.85) and R_f 0.85 in Solvent 2 in the SCP1⁺ chromatogram. *b*, Bioautography of the chromatograms shown in *a*. Chromatograms were laid on a basal layer of nutrient agar in a 23 × 23 cm Bio-Assay plate (A/S Nunc, Roskilde, Denmark) and overlaid with molten nutrient agar containing *Bacillus mycoides* (3 ml of late log-phase culture per 100 ml agar) and 0.0012% 2,3,5-triphenyl tetrazolium chloride. The plate was incubated overnight at 36 °C. Note the zone of inhibition of *B. mycoides* by the ultraviolet-absorbing spot in the SCP1⁺ chromatogram (left). Nonspecific inhibition by material accumulating at the solvent fronts is present in both preparations.

strains apparently having the plasmid in the autonomous state (SCP1$^+$) transfer the SCP1$^+$ characteristics independently of chromosomal markers ('infectiously') to SCP1$^-$ strains[8], second, SCP1$^+$ strains produce SCP1$^-$ strains with frequencies much higher than those expected of mutations[9], while SCP1$^-$ strains do not revert to SCP1$^+$; third, certain strains, called SCP1' by analogy with F' strains of *Escherichia coli* K12, transfer particular chromosomal genes infectiously, together with the SCP1$^+$ characteristics, to SCP1$^-$ strains[10], as expected if such chromosomal genes have become inserted into the plasmid; fourth, other strains transfer characteristic patterns of chromosomal markers to SCP1$^-$ strains; these are interpretable as having SCP1 inserted into the chromosome at various points[11].

SCP1$^+$ strains (then called IF) were described as producing a diffusible inhibitor that arrested the development of SCP1$^-$ cultures (called UF); SCP1$^+$ cultures were resistant to this inhibitor[8]. We now show that this material is an antibiotic (rather than, for example, a bacteriocin or bacteriophage) and that several genes controlling its biosynthesis, as well as resistance to it, are borne on the SCP1 plasmid.

since it diffuses through dialysis membranes. Two-dimensional chromatography of extracts of culture fluids reveals a single difference between isogenic SCP1$^+$ and SCP1$^-$ cultures: the presence, in SCP1$^+$ preparations, of a discrete ultraviolet-absorbing spot (Fig. 1*a*) which produces a zone of inhibition of the test organism in bioautography (Fig. 1*b*). Antibiotic eluted from this spot inhibited sensitive organisms at concentrations less than 10 μg ml^{-1}.

Seven strains carrying SCP1-linked mutations resulting in lack of antibiotic activity were isolated from a strain bearing the SCP1'-*cysB*$^+$ plasmid[10]. They were detected by replica plating colonies to plates spread with SCP1$^-$ spores and occurred, after mutagenesis by ultraviolet (254 nm), or by 365 nm light in the presence of 8-methoxypsoralen[7], with a frequency of about 5 × 10^{-4}. All the mutants retained antibiotic resistance. Most were revertible to antibiotic production, suggesting that they were point mutations. Plasmid linkage of the mutations was demonstrated by transferring the plasmid from each mutant to a *cysB*$^-$ SCP1$^-$ strain, which remained antibiotic negative, while acquiring antibiotic resistance and the extrachromosomal *cysB*$^+$ allele.
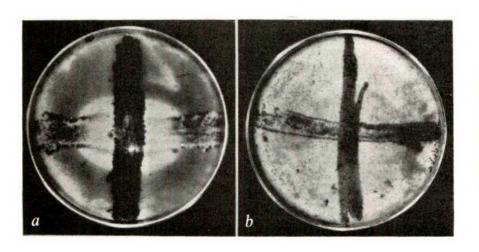
Fig. 2 Tests for antibiotic cosynthesis by pairs of antibiotic-negative mutants. Each plate was prepared by inoculating two mutants, by replica plating from master plates, at right angles on a plate sown with spores of an SPC1$^-$ tester strain. *a*, Cosynthesis by R40 (secretor: vertical streak) and R39 (convertor: horizontal streak): a zone of inhibition of the test strain extends from the centre of the plate along the R39 culture. *b*, Lack of cosynthesis by R40 (vertical streak) and R53 (horizontal streak).

When spores of SCP1$^+$ and SCP1$^-$ strains are inoculated close together on agar, both cultures reach a stage immediately preceding aerial mycelium production before antibiotic diffusing from the SCP1$^+$ culture prevents further development of the nearby SCP1$^-$ culture. Production of antibiotic by the SCP1$^+$ culture, rather than susceptibility of the SCP1$^-$ culture to its action, is correlated with the development of aerial mycelium; young vegetative mycelium of SCP1$^-$ strains is susceptible to antibiotic produced by mature SCP1$^+$ cultures as can be shown by placing agar plugs cut from such cultures on plates inoculated with SCP1$^-$ spores. Susceptibility to the antibiotic is not confined to SCP1$^-$ cultures of *S. coelicolor* A3(2); the antibiotic has a wide antibacterial spectrum. About half of a collection of *Streptomyces* spp. was found to be susceptible[12]. Of other actinomycetes and related bacteria, one *Nocardia* sp. was sensitive and two *Mycobacterium* spp. and one *Corynebacterium* sp. were resistant. Members of all Gram-positive eubacterial genera tested were sensitive: *Azotobacter*, *Bacillus* (six species), *Micrococcus*, *Staphylococcus*, *Streptococcus*. One member of each of the Gram-negative genera *Escherichia*, *Klebsiella*, *Salmonella*, *Serratia*, *Agrobacterium*, *Pseudomonas* and *Rhizobium* was resistant, but *Erwinia carotovora* and *Proteus vulgaris* were susceptible. Fungi of the genera *Saccharomyces* and *Penicillium* were unaffected.

The antibiotic is of comparatively low molecular weight,

Pairs of mutants were tested for antibiotic cosynthesis[13] by inoculating them at right angles on plates sown with spores of an SCP1$^-$ tester strain. Positive tests (Fig. 2*a*) indicated secretion of a material without antibiotic activity by one strain and its conversion to antibiotic by the other; the shape of the inhibition zone allowed identification of the secretor and convertor mutants. The simplest explanation is that the mutations are in plasmid-linked structural genes controlling a pathway of antibiotic synthesis, rather than genes indirectly concerned in antibiotic production. Four plasmid-coded biosynthetic steps are indicated by the classification of the seven mutants into four cosynthetic classes; doubtless this is an underestimate of the number of steps in the pathway, although some steps yet to be revealed by mutants may be controlled by chromosomal genes.

The mechanism of SCP1-coded antibiotic resistance is unknown; however, the finding that a *Streptomyces* plasmid codes for synthesis of and resistance to the same antibiotic is interesting in relation to the possible origin of plasmid-borne genes conferring resistance to actinomycete antibiotics in Gram-negative eubacteria. The theory that such genes originated in antibiotic-producing actinomycetes, many of which possess antibiotic-inactivating enzymes analogous with those of plasmid-carrying Gram-negative eubacteria[14], does not require such enzymes to be coded for by plasmids in the producing organisms. But transfer to other genera might have been aided if this were the case, a

possibility made more likely by our results with *S coelicolor*

A second implication of these results is in the evaluation of techniques of gene amplification and other aspects of plasmid manipulation in developing new strains of industrial antibiotic-producing actinomycetes

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The β₂-microglobulin gene is on chromosome 15 and not in the HL-A region

POSSIBLE evolutionary homology between the genetic regions controlling histocompatibility antigens (such as HL-A in man and H-2 in the mouse) and immunoglobulins has been proposed[1][2] Recent studies involving β_2-microglobulin (β_2m) seem to support this idea Human β_2m was found originally in the urine of patients with renal tubular dysfunction It is also present in serum and on the surface of most types of cell[3][4] It has a molecular weight of about 12,000 and shows substantial sequence homology with the constant region domains of immunoglobulin heavy chains[5][6] Partially purified papain[7][8] and detergent[9] solubilised HL-A molecules, and H-2 molecules[10], consist of two chains one of which is invariant and has been identified as β_2m[11-13] Chance association during the isolation procedure has been shown to be unlikely by the cocapping on the cell surface of β_2m with the allogeneic chain of HL-A that carries the usual serologically detected determinants[14-16]

The genes controlling the HL-A determinants form part of a complex genetic region associated with immune functions including transplantation rejection, mixed lymphocyte culture stimulation, graft *versus* host response, cell mediated lympholysis, immune response and some complement functions (see ref 17) The possibility that all these functions, which involve the cell surface and its role in recognition, are mediated by molecules evolutionarily related to immunoglobulins might be supported by the location of the gene for β_2m in this region Using the techniques of somatic cell genetics with human–mouse hybrid cells we have, however, shown that the gene for β_2m is not located in the HL-A region

Antiserum to human β_2m produced in rabbits (Dakopatts, Denmark) was active in a cytoxicity assay performed using our standard procedure[18] To remove nonspecific toxicity against mouse cells specially selected rabbit complement was absorbed with the 8-azaguanine L cell derivative 1R, which was the parent mouse cell in most of the hybrids tested

The last dilution of the antiserum which gave a 50% kill in complement-dependent cytotoxicity on primary human fibroblasts was 1/64 The antiserum did not kill the parent

Table 1 Segregation of β₂m and other human markers in a series of human–mouse hybrids

Hybrid	β₂m	Chromosome 15* MPI/PK	Chromosome 11*† SA-1	Chromosome Xŧ G6PD	Karyotyping carried out
1W1§	−	−	±	+	Yes
2W1§	−	−	−	+	Yes
4W10§	−	−	+	+	Yes
Subclones¶ G1, G6, G10	−	−	+	−	Yes
4W7§ Subclone ‖ R18	−	−	+	−	No
P7Aŧŧ	−	ND	−	−	Yes
4 12 Z§	−	−	+	+	No
HORL 1,2 and 9§††	+	+	+	+	Yes
HORL 8	+	+	−	+	No
HORL 11	+	+	+	+	No
HORP§††	+	+	+	+	No
Subclones 24, 25	+	+	+	+	Yes
14	−	−	+	+	Yes
27R	+	+	+	−	Yes
3W4§ Subclone** DF42	+	+	+	−	No
DUR¶¶ 4, 5, 10	+	+	+	+	Yes
DUR¶¶ 4R, 5R, 10R	−	−	+	−	Yes
AD§§	−	+	+	+	Yes

* Chromosome 15 markers assayed for as described ref 23
−, Absence, +, presence in a majority of cells, ND, Not done, ±, presence in a small proportion of cells
† Chromosome 11 linked antigen SA-1 as described in ref 30, LDHA as described in ref 31 Either was taken to indicate the presence of chromosome 11
ŧ Chromosome X G6PD assayed for as described in ref 32
§ Hybrids between normal peripheral blood lymphocytes and 1R See refs 31, 32 and 33
¶ 8-Azaguanine selected derivatives of 4W10
‖ 8-Azaguanine selected derivatives of 4W7
** 8-Azaguanine selected derivatives of 3W4
†† HORL and HORP hybrids and subclones are described in detail in ref 23
ŧŧ Described in ref 34
§§ See text and ref 25
¶¶ The DUR hybrids were made between 1R and a human X/15 translocation fibroblast in which the X/15 chromosome is active Selection in 8-azaguanine resulted in the loss of both chromosome X and 15 markers Further details of the analysis of these hybrids will be published elsewhere

mouse 1R cells but had a titre of 1/32 on a representative positively reacting human–mouse hybrid, HORL9 The specificity of this reaction on hybrid cell lines was checked by absorptions with different cells and with partially purified β_2m Absorption with mixed human type A and O red blood cells[19] (three times with an equal volume), 1R (two times with 2×10^8 cells ml⁻¹) or spleen cells taken from CBA and C3H mice, the strain from which L cells are derived (two times with 5×10^8 cells ml⁻¹) failed to reduce the titre of the antiserum on primary human fibroblasts or the hybrid, HORL9 In contrast, the antiserum's activity on either fibroblasts or the hybrid could be totally absorbed by HORL9 (two times 2×10^8 ml⁻¹), the human lymphoblastoid cell line T51 (2×10^8 ml⁻¹) and 100 μg ml⁻¹ of partially purified β_2m The β_2m (a gift from Professor B Pernis) was prepared from the urine of crash victims, the material showed five equally staining bands by SDS electrophoresis A more highly purified preparation prepared from urine by D Snary and M Crumpton, showing only one major band and two minor bands on SDS electrophoresis, also completely absorbed the antiserum at a concentration of about 20 μg ml⁻¹ On Ouchterlony double diffusion plates both preparations gave a single precipitin line with the Dakopatts antiserum The antiserum absorbed with 4×10^8 1R cells ml⁻¹

gave this precipitin line while absorption with 4×10^8 HORL9 cells ml^{-1} removed it A goat antiserum to human β_2m (Kallestad) gave a reaction of total identity with the Dakopatts antiserum on Ouchterlony plates against partially purified β_2m It also gave identical cytotoxic reactions with the Dakopatts antiserum

The cytotoxicity reactions were confirmed using immunofluorescence 1×10^5 test cells were mixed with 50 μl of a suitable dilution (1/50) of test antiserum, after 30 min the cells were washed three times and suspended in 50 μl of a 1/20 dilution of fluorescein-labelled sheep anti-rabbit IgG (Wellcome) The cells were incubated at 4 °C 30 min, washed three times and mounted in 50% glycerol in phosphate buffered saline on a slide under a sealed coverslip The cells were then scored under a Leitz orthoplan fluorescence microscope fitted with a ploem epiilluminator, a BG12 primary filter, K510 secondary filters and a K495 suppression filter Species specificity in immunofluorescence was only achieved after absorption with 2×10^8 1R cells ml^{-1} Further absorption with 1R or human red blood cells failed to remove any activity on human lymphoid cells or the hybrid HORL9 As in cytotoxicity, the reaction in immunofluorescence could be totally absorbed by the human lymphoid cell T51

The specificity of the anti-human β_2m was further checked by immunoprecipitation of membrane extracts Incubation[20] of chloramine T ^{125}I-labelled deoxycholate-solubilised membranes[21] from a human lymphoblastoid cell line with rabbit anti-β_2m, followed by the addition of horse anti-rabbit IgG, could be used to precipitate a low molecular weight protein of the same size as β_2m, identified on SDS polyacrylamide gels A similar precipitation procedure with mouse spleen cell membranes did not yield this protein It has been suggested that rabbit anti-human β_2m (ref 22) can recognise mouse lymphocytes, causing B cell mitogenesis These authors agree with us, however, that the antiserum is not cytotoxic for mouse cells

The results of testing the anti-β_2m sera by complement-dependent cytotoxicity and immunofluorescence, as described above, on a series of human–mouse hybrids are given in Table 1 The two techniques used gave identical results Work in our laboratory[23] using human–mouse somatic cell hybrids has provided evidence that the genes for mannose phosphate isomerase (MPI, EC 5 3 1 8) and pyruvate kinase 3 (PK-3, EC 2 7 40) can be assigned to chromosome 15 Our study of these and other hybrids, as well as providing more data on this linkage, provides evidence that β_2m is also coded for by a gene on chromosome 15 The origin of most of the hybrids studied and the techniques for their production, propagation and chromosomal analysis were as previously described (see refs in Table 1 and ref 24)

The β_2m positive hybrid HORL9 has only three human chromosomes[24], X, 11 and 15 HORP27R, on the other hand, is an 8-azaguanine-resistant hybrid derivative which lacks the X chromosome, but is β_2m positive On the basis of these data, β_2m must be coded for by a gene on either chromosome 11 or 15 HORL8, however, which lacks chromosome 11 and is β_2m positive excludes linkage to chromosome 11 From Table 1 it can be seen that four primary hybrids with chromosome 15 have β_2m and six primary hybrids lacking chromosome 15 lack β_2m The corresponding subclone numbers are 12 (+ +) and 8 (− −) The only exception to the expression of human β_2m in human–mouse hybrids with chromosome 15 is AD, which comes from a cross of the human lymphoblastoid line Daudi by the mouse L cell derivative A9 (ref 25) This exception agrees with the fact that Daudi does not express β_2m[4], which we have confirmed This suggests that the lack of expression of β_2m in Daudi may be due to a change in the genetic region encompassing the β_3m gene, a possibility which can be checked by analysis of more clones derived from Daudi $\times$ A9 hybrids

The only simple conclusion consistent with these data is that β_2m is coded for by a gene located on chromosome 15 We have obtained further confirmation of this assignment by the use of anti-β_2m and complement to select for hybrids which have lost the chromosome carrying the β_2m gene

It should be noted that the fact that HORL9 absorbs out all the activity of the Dakopatts antiserum on human fibroblasts, together with the lack of reactivity of this serum on hybrids containing human chromosomes X and 11 without 15, is strong evidence for the antiserum recognising only a product of chromosome 15, both on human–mouse hybrids and on normal human cells This product is human β_2m as shown by the absorptions with partially purified β_2m

Population and family studies[26] as well as inter[27] and intraspecific[28] hybrids have been used to assign the complex HL-A region to chromosome 6 The two polypeptides of the HL-A molecule are therefore coded for by unlinked genes This result does not, of course, necessarily imply that the genes for the allogeneic chain of the HL-A molecule are not related to the β_2m gene The light and heavy chain genes of the immunoglobulins, which are evolutionarily related, are unlinked[29] Our data do, however, raise the question of how many genetic regions exist in the human genome whose evolution can be traced to a common ancestry with the immunoglobulin genes The possibility that the immunoglobulin heavy chain genes are linked to the β_2m gene on chromosome 15 has not yet been excluded

It is interesting that none of the hybrids in Table 1 has chromosome 6 nor can they be killed by HL-A antisera in a normal cytotoxicity test Hybrids derived from HORL9 with chromosome 15 as the only human chromosome present can express β_2m, strongly suggesting that human β_2m can be expressed on cells in the absence of the products of the human major histocompatibility region We are now using our hybrids to study the possible interaction between mouse H-2 allogeneic chains and human β_2m In collaboration with R Kennett, we have recently detected HL-A on human–mouse hybrids by direct cytotoxicity and absorption in the presence of human β_2m

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Genes required for cytotoxicity against virus-infected target cells in *K* and *D* regions of H-2 complex

EVIDENCE is mounting that in mice, certain specific immunological effector functions of thymus-derived (T) lymphocytes are efficient only when donors of T cells and the cells with which they interact have at least a part of the H-2 gene complex in common[1-8] Examples include T helper function *in vivo* and *in vitro*[1-3], cytotoxicity mediated by T cells against virus-infected[4-6] or TNP-modified[7] target cells *in vitro*, immunopathology mediated by T cells[4], or protection against bacterial[8] and viral infection[6] *in vivo* This requirement for H-2 compati-

bility has been studied in detail by measuring the cytotoxicity of T cells from donors immunised with either lymphocytic choriomeningitis (LCM) or ectromelia virus using target cells infected with the homologus virus

These two viruses are very different, LCM being a non-cytopathic, single-stranded RNA virus which acquires an envelope by budding from infected cell membranes, whereas ectromelia is a cytopathic DNA virus which is assembled completely within infected cells[9] Evidence described in detail elsewhere[4,10,11] indicates that immune T cells kill virus-infected target cells directly by contact without production of detectable soluble factors or any requirement for ancillary cells such as mononuclear phagocytes or thymus-independent (B) cells Experiments with target cells of different H-2 haplotypes and a repertoire of inbred mouse strains, including congenic pairs, have shown that the non-H-2 genetic background, the M locus and H-2 public specificities are irrelevant[4,11] Maximal cytolysis only occurs when immune T cells and virus-infected target cells share the same H-2 haplotype[4,11]

These findings suggest that the genes required are either the H-2 private specificities, which code for the major H-2 antigens on which the haplotype classification is based, or genes closely

Table 1 H-2 composition of mouse strains used

Strain	H-2 regions					
	K	A	I B	C	S	D
B10 A	k	k	k	d	d	d
B10 A(2R)	k	k	k	d	d	b
B10 A(4R)	k	k	b	b	b	b
A TL	s	k	k	k	k	d
A TH	s	s	s	s	s	d
C3H OH	d	d	d	d	d	k
C3H OL	d	d	d	d	k	k
AQR	q	k	k	d	d	d

linked to them in the *K* and *D* regions of the H-2 complex Identity of *Ir* genes, which map in the *I* region between *K* and *D* (Table 1) and which are common to many different H-2 haplotypes, should not be sufficient[12] This reasoning was tested directly using mouse strains bearing recombinant H-2 haplotypes (Table 1) Immune spleen cells were obtained from donor mice immunised with either LCM virus or ectromelia virus as

Table 2 Activity of splenic cytotoxic T cells* from LCM-immunised mice against target cells infected with LCM virus

Mouse strain†	Spleen cell status	L929 targets (H-2^k)		P-815 targets (H-2^d)		Mouse embryo cells (H-2^b)	
		Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
B10 A	Normal	15 9 ± 1 0	19 4 ± 1 3	21 4 ± 1 4	30 1 ± 3 2	36 8 ± 1 6	45 1 ± 1 4
	Immune	17 2 ± 1 7	72 6 ± 0 9	31 2 ± 2 3	86 5 ± 1 2	38 5 ± 2 9	45 9 ± 1 8
B10 A(2R)	Normal	24 9 ± 0 7	20 9 ± 1 8	22 7 ± 1 1	29 0 ± 3 7	40 1 ± 0 7	48 2 ± 0 8
	Immune	21 9 ± 2 0	80 9 ± 2 6	41 7 ± 0 9	49 0 ± 1 3	42 7 ± 2 1	61 9 ± 1 8
B10 A(4R)	Normal	17 0 ± 0 8	18 5 ± 2 1	32 1 ± 3 2	26 0 ± 4 5	39 6 ± 1 0	45 7 ± 2 0
	Immune	18 9 ± 1 3	74 3 ± 1 6	40 7 ± 1 9	35 9 ± 1 7	44 4 ± 1 4	59 7 ± 1 5

Mouse macrophages, SJL (H-2^s)

Mouse strain†	Spleen cell status	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
A TL	Normal	14 1 ± 0 6	16 5 ± 0 9	14 3 ± 0 9	14 1 ± 1 1	40 7 ± 4 2	39 1 ± 3 7
	Immune	17 6 ± 1 7	17 6 ± 1 7	16 2 ± 1 1	64 4 ± 2 4	39 6 ± 2 4	76 1 ± 2 9
A TH	Normal	12 3 ± 0 7	13 0 ± 0 9	30 5 ± 4 5	28 6 ± 2 1	ND	ND
	Immune	13 2 ± 1 6	16 3 ± 1 9	30 1 ± 4 8	81 0 ± 1 2	ND	ND
C3H OH	Normal	20 8 ± 0 6	15 4 ± 1 4	14 1 ± 0 5	27 8 ± 1 1	ND	ND
	Immune	22 4 ± 1 1	56 5 ± 2 0	23 7 ± 0 5	74 5 ± 2 8	ND	ND
C3H OL	Normal	23 2 ± 0 6	27 4 ± 0 6	15 3 ± 0 4	17 2 ± 0 8	ND	ND
	Immune	52 8 ± 2 2	80 5 ± 1 3	18 0 ± 0 3	49 0 ± 0 8	ND	ND

Mouse macrophages, DBA/1 (H-2^q)

Mouse strain†	Spleen cell status	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
AQR	Normal	ND	ND	16 1 ± 0 5	16 9 ± 0 6	39 1 ± 3 5	36 9 ± 2 6
	Immune	ND	ND	28 0 ± 0 5	92 6 ± 0 6	44 8 ± 2 0	62 6 ± 2 3

*Expressed as percentage ⁵¹Cr released (mean of four replicates ± s e m) over 16 h at a killer–target ratio of 30 1 (corrected for water lysis) Significant specific lysis (P < 0 05) is indicated by italics
†Data given in this table were derived from several separate experiments in which the various mouse strains were tested CBA H (H-2^k), BALB/c (H-2^d), C57BL (H-2^b), SJL (H-2^s) and DBA/1 (H-2^q) mile were always included in the experiment as controls where necessary They gave specific lysis only with H-2-compatible infected target cells (see refs 4 and 9) (data not shown)
ND, not determined

Table 3 Activity of splenic cytotoxic T cells* from ectromelia-immunised mice against target cells infected with ectromelia virus

Mouse strain†	Spleen cell status	L929 targets (H-2^k)		P-815 targets (H-2^d)		Mouse embryo cells (H-2^b)	
		Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
B10.A	Normal	28 2 ± 0 5	28 3 ± 0 5	17 2 ± 0 6	29 6 ± 0 3	34 7 ± 1 5	34 1 ± 1 0
	Immune	40 5 ± 0 3	*95 5 ± 1 3*	24 6 ± 1 1	*59 4 ± 1 2*	34 1 ± 1 6	32 9 ± 1 0
B10 A(2R)	Normal	25 6 ± 2 3	26 7 ± 1 7	22 0 ± 1 5	24 0 ± 1 6	38 1 ± 1 8	34 5 ± 1 7
	Immune	29 7 ± 1 2	*83 4 ± 1 9*	27 0 ± 1 6	*37 8 ± 3 1*	41 7 ± 1 2	*67 1 ± 1 3*
B10 A(4R)	Normal	25 5 ± 1 2	27 4 ± 2 3	28 6 ± 3 0	23 6 ± 0 8	45 5 ± 2 0	34.7 ± 1 1
	Immune	30 1 ± 1 2	*78 5 ± 2 7*	31 1 ± 4 0	30 5 ± 3 5	40 3 ± 1 9	*58 8 ± 0 9*
A TL	Normal	35 6 ± 1 3	31 5 ± 1 4	19 5 ± 0 2	23 7 ± 0 5	ND	
	Immune	42 3 ± 1 7	44 0 ± 1 5	32 3 ± 0 7	*78 4 ± 0 6*	ND	
A.TH	Normal	36 1 ± 1 2	38 0 ± 2 1	20 1 ± 0 3	22 1 ± 0 4	ND	
	Immune	39 0 ± 0 8	37 4 ± 1 2	21 6 ± 0 5	*63 1 ± 1 3*	ND	
C3H OH	Normal	21 3 ± 1 5	21 2 ± 1 1	17 4 ± 0 6	27 4 ± 1 2	ND	
	Immune	33 2 ± 2 0	*47 0 ± 2 2*	37 1 ± 0 7	*71 5 ± 3 8*	ND	
C3H OL	Normal	32 0 ± 2 9	33 1 ± 2 8	20 6 ± 2 6	22 5 ± 2 1	ND	
	Immune	27 2 ± 2 1	*50 9 ± 1 7*	39 0 ± 1 5	48 0 ± 2 8		

Mouse macrophages, DBA/1 (H-2^q)

AQR	Normal	25 8 ± 2 2	20 8 ± 1 0	15 4 ± 0 8	20 9 ± 1 4	39 3 ± 1 8	42 5 ± 3 7
	Immune	39 9 ± 2 1	24 8 ± 0 8	31 5 ± 1 2	*58 9 ± 4 1*	43 4 ± 3 6	*65 0 ± 4 4*

*Expressed as percentage [51]Cr released (mean of four replicates ± s. e m) over 16 h at a killer–target ratio of 60 1 (corrected for water lysis) Significant specific lysis ($P < 0.05$) is indicated by italics

†Data given in this table were derived from several separate experiments which included control strains listed in the footnote to Table 2 ND, not determined

described elsewhere[5,11], and specific cytotoxicity mediated by T cells was measured by [51]Cr release from virus-infected target cells of various H-2 haplotypes using optimal spleen cell–target cell ratios[5,11] (Tables 2 and 3) Immune cells almost invariably caused more lysis than normal cells, irrespective of target cell type, but significant specific lysis was defined as occurring only when combinations of immune cells and infected targets gave [51]Cr release which was significantly higher ($P < 0.05$) than all three control combinations, such as immune cells with uninfected targets, or normal cells with either infected or uninfected targets Comparison of Tables 1 and 2 shows that specific lysis of LCM-infected H-2^k target cells required immune T cell donors to be of H-2^k type only in the K or D regions of the gene complex It was not sufficient for all of the I and S region to be k (as in A TL mice) Lysis of LCM-infected H-2^d or H-2^b targets also required only D region homology The use of H-2^s and H-2^q macrophages as target cells confirmed that K region homology was sufficient

Results obtained with ectromelia virus (Table 3) were essentially similar to the LCM system, with one exception Lysis of infected H-2^k target cells by ectromelia-immune cells from C3H OH or C3H OL mice (which are of H-2^k type in the D region) was not as reproducible or powerful as with LCM Ectromelia provoked a significant response against infected H-2^k targets (Table 3) in only one out of three experiments with C3H OH, and one out of two experiments with C3H OL, whereas a significant response always occurred against infected H-2^d targets Thus major gene(s) active in the LCM system seemed less active in the ectromelia system The factors responsible for this variation are not known, but Ir genes are candidates for further investigation

With both viruses, B10 A (2R) gave a small but statistically significant response against H-2^d target cells in one out of two experiments (Tables 2 and 3), suggesting that genes outside the K or D regions (for example, in IC or S regions in this case) may sometimes exert a minor influence

In summary, these data support the concept that the major genes required for cytolysis mediated by T cells, of virus-infected target cells are located in the K or D regions of the H-2 complex In most cases, these genes are sufficient, without the requirement for I region homology Whether Ir genes play a secondary, regulatory role remains to be determined This is consistent with the hypothesis proposed[4,5] that the H-2-dependent restriction of lysis, mediated by T cells, of virus-infected target cells results from T cell recognition of altered self antigens (possibly H-2 private specificities) on the surfaces of virus-infected cells

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Evolutionary conservation of H-Y ('male') antigen

The male specific (H-Y) antigen of mice was discovered with the observation that within certain inbred strains, females reject male skin grafts, whereas skin grafts exchanged between all other sex combinations are accepted[1] (reviewed in ref 2) It is now established that females sensitised with male skin grafts (or immunised with male spleen cells) produce antibody which is cytotoxic for sperm[3] and dissociated male epidermal cells[4] Using the sperm cytotoxicity test and the mixed haemadsorption-hybrid antibody (MHA HA) test, we demonstrated earlier[5] that the H-Y antigen of mice is cross reactive or identical with antigen
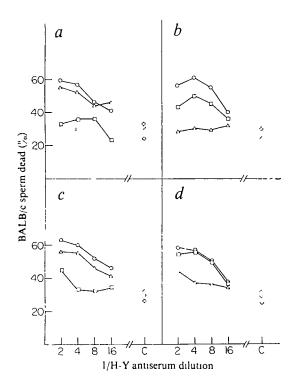
Fig 1 Summary of cytotoxicity tests on mouse sperm with mouse H-Y antiserum after absorption with male or female cells from four test species Each point is an average of values from three separate tests (b, chicken and c, leopard frog), four tests (a, mouse) or six tests (d, X laevis) O, Unabsorbed H-Y antiserum, △, absorbed with female cells, □, absorbed with male cells (spleen cells of mouse, blood cells of chicken, spleen and liver cells of amphibians), ◇, control, complement included, antiserum omitted H-Y antisera were prepared in adult C57BL/6 female mice by four to six weekly intraperitoneal injections of 30–60 x 10⁶ C57BL/6 male spleen cells Individual serum samples were tested, diluted one-half and pooled for absorption Cell suspensions for absorption were prepared as follows (1) Mouse spleen, according to Wachtel et al[5] (2) Amphibians spleen and liver were minced in Earle's balanced salt solution (EBSS) and the supernate, containing free cells in suspension, was removed and centrifuged at 100–150g for 10 min The cells were washed twice and resuspended in EBSS (3) Chicken red blood cells and buffy coat leukocytes were collected from whole heparinised blood after centrifugation at 700–800g 10 min Cells were washed twice and resuspended in EBSS All absorptions were carried out on ice for 30–40 min, 1–2 parts serum per 1 part cells Epididymal sperm, prepared from BALB/c mice according to Goldberg et al[3], were incubated with H-Y antisera and with absorbed rabbit serum diluted 1 14 (complement source) for 45–50 min Trypan blue dye was added during the last 5–10 min of incubation and the tests read in a haemocytometer Dead sperm stained with the dye

found in male rats, guinea pigs, rabbits and humans Since then we have extended our survey to classes other than mammals, and we give evidence here for the occurrence of murine H-Y (or a cross reactive) antigen in the white leghorn chicken (*Gallus domesticus*) and in two amphibian species, the leopard frog (*Rana pipiens*) and the South African clawed frog (*Xenopus laevis*)

H-Y antisera were raised in female C57BL/6 mice by immunisation with male C57BL/6 spleen cells as reported

Table 1 Sex association of H-Y antigen

Class	Species	Heterogametic sex	H-Y antigen found in
Mammalia	*Mus musculus**	Male	Male
Aves	*Gallus domesticus*	Female	Female
Amphibia	*Rana pipiens*	Male	Male
	Xenopus laevis	Female	Female

* Mouse

previously[5] For each serological test, the selected H-Y antiserum pool was diluted and divided into three parts (as in our earlier studies[5]) The first part was absorbed with female cells of the species being tested (aliquot A) and the second, with male cells (aliquot B) while the third was not absorbed (aliquot C) The three aliquots were then titrated for residual cytotoxic activity against mouse sperm Absorption of cytotoxic activity from aliquot A or B would indicate that H-Y antibody must have been removed specifically by female (A) or by male (B) cells of the species being tested, signifying that the absorbing cells of this species carry a surface component identical or cross-reactive with mouse H-Y antigen

H-Y antiserum aliquots used in the confirmatory MHA HA tests were prepared and absorbed in the same way The indicator cells, mouse sperm, were then incubated with the three aliquots A, B and C, washed, and subsequently exposed to a rabbit hybrid antibody with the specificity anti-mouse Ig / anti-sheep red blood cells (SRBC) The sperm were again washed and then reacted with SRBC In this assay, sperm which have taken up H-Y antibody, and therefore also the hybrid antibody, bind SRBC to form rosettes Absorption of H-Y antibody from

Table 2 Summary of MHA HA test data reaction of mouse H-Y antiserum with mouse sperm after absorption with cells from male or female chicken or X laevis

| | Serum aliquot | | |
	A (absorbed ♀)	B (absorbed ♂)	C (unabsorbed)
Chicken	14 2*	23 8	25 4
X laevis	7 0	15 9	16 6

To avoid repeated centrifugation, the MHA HA reactions were carried out by centrifuging the sperm through a discontinuous density gradient containing the reagents and wash layers in sequence[5,18] Gradients were established in tubes made from 1-ml disposable glass pipettes by addition of heat-inactivated, gamma globulin-free foetal bovine serum (FBS) The concentration of FBS in each layer was adjusted to prevent intermixing Sperm were obtained from the epididymis of BALB/c mice according to Goldberg et al[3] A sample of 0 05 ml of a suspension containing 5–10 x 10⁶ sperm per ml was reacted with 0 05 ml H-Y antiserum diluted one-half, the suspension of sensitised sperm was then placed in the gradient tube and centrifuged at 35–50g for 10 min and at 150–200g for 10 min The pellet (containing sperm and SRBC) was removed and recentrifuged (35–50g for 10 min) to enhance rosette formation After standing for 30 min on ice, the pellet was resuspended and samples were read in a haemocytometer Three tests each (chicken and X laevis) were scored as coded samples by an observer who recorded the counts of rosettes and free sperm The values (percentages) shown here were derived from the formula number of rosettes / number of rosettes+free sperm Any sperm cell to which three or more sheep red blood cells had adsorbed was scored as a rosette

* Average number of rosettes per 100 sperm cells

aliquot A or B in the MHA HA test is indicated by a decrease in the number of rosettes in comparison with the number observed with unabsorbed antiserum (aliquot C)

In view of existing evidence[6,7] for a sex-associated transplantation antigen in the heterogametic sex of the chicken (which is the female in this case, Table 1) we studied this species first, using both the cytotoxicity and MHA HA tests The results of our experiments (Fig 1, Table 2) indicate that the chicken has an H-Y (H-W) antigen identical or cross reactive with mouse H-Y, and that it is expressed by the female The decrease in cytotoxicity titre produced by absorption with male chicken cells (Fig 1) is not more than what can be attributed to anti-complementary effects of the absorption procedure Such a decrease was not seen in the MHA HA test, which does not depend on complement (Table 2)

Since birds and mammals represent widely divergent pathways of reptilian evolution, we suspected at this point

that H-Y antigen would be found in reptiles, and so we next investigated its possible occurrence in Amphibia, a more primitive class of vertebrates In *R pipiens* the male is heterogametic as in mammals whereas in *X laevis* the female is heterogametic as in birds (Table 1) Our serological tests (Fig 1 and Table 2) demonstrate that cells from both these species contain a surface component identical or cross reactive with H-Y antigen of the mouse Moreover, the antigen is found in the heterogametic sex of both these amphibian species Thus it would seem that the cell surface component conferring H-Y antigenicity in the mouse has been conserved through some 300 Myr of evolution (spanning the Carboniferous radiation of amphibians and the Pleistocene emergence of man)

Although the phylogenetic conservation of a particular polypeptide sequence (indicated here by common antigenicity) suggests some essential or highly advantageous role, the reason for the conservation of H-Y antigen is not at all obvious Nevertheless the association of H-Y antigen with either one sex or the other in those species tested so far seems to indicate a sex-related function of some kind, perhaps one concerned with the recognition of one sex by the other in nature (see discussion by Thomas[8])

As to whether or not H-Y antigen expression is hormone dependent[9], several experiments involving male←→female transplantation and/or hormonal manipulation have given conflicting results[10-16] Sex-limited, hormone-dependent traits affecting the cell surface are known however Indeed the 'Hi' red blood cell agglutinogen of chickens is normally confined to females although this trait can be induced in males by treatment with diethylstilboesterol[17] Thus it seems wise to leave open the question of the extent to which expression of H-Y antigen may be influenced quantitatively by sex hormones, although our finding that H-Y may occur in females implies that its expression is not qualitatively hormone-dependent In this context it remains to be determined whether or not the *H-Y* structural locus is situated on the Y chromosome An exceptional species in which H-Y antigen were found in the homogametic sex would suggest that the *H-Y* structural locus is autosomal (or even X-linked) We are now investigating what may prove to be such an exception

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Production of lymphoid tumours in hamsters by direct implantation of normal human leukocytes

LEUKOCYTES from patients with various lymphoproliferative diseases can be serially transplanted as malignant lymphoid tumours in immunosuppressed newborn hamsters[1] We report that normal human leukocytes can also be transplanted into newborn hamsters treated with antilymphocyte serum

Three human leukocyte samples were used, one from peripheral blood of an Epstein–Barr virus (EBV) antibody-positive normal male and two from umbilical cord blood with and without EBV infection Leukocytes were separated by Dextran sedimentation from 20 ml of peripheral or cord blood and $0 5-1 \times 10^7$ viable leukocytes were implanted intraperitoneally into Syrian golden hamsters less than 24 h of age This was followed by twice weekly intraperitoneal inoculation of $0 1$ ml rabbit antilymphocyte serum against hamster thymocytes The leukocytes from cord blood were incubated with 1 ml of a filtered freeze–thaw extract of the (EBV+) B95 cell line[2] at 37 °C for 2 h, before implantation

All three hamsters transplanted with peripheral leuckocytes and two out of three hamsters transplanted with cord leukocytes infected with EBV were found to have lymphoid tumours involving the lymph nodes, liver, lungs and kidneys, when killed on days 11–21 No tumours were observed, however, in hamsters transplanted with cord leukocytes not infected with EBV

Enlarged inguinal lymph nodes resulting from transplantation of peripheral leukocytes or EBV-infected cord leukocytes were cultured in medium RPMI 1640 supplemented with 20% foetal calf serum, and two lymphoblastoid cell lines were established from a hamster of each group The cells began to grow vigorously in about 3–4 weeks and have been maintained for 6 months These cell lines have a normal diploid human chromosome constitution and are positive for EBV nuclear antigen[3] Electron microscopy revealed EBV particles in lymphoid cells

Our observation seems to be analogous with the establishment of lymphoblastoid cell lines *in vitro* from normal human peripheral and cord leukocytes, for which an essential role of EBV has been demonstrated[4,5] Successful heterotransplantation of normal human leukocytes into hamsters may be based on the same mechanism as in the *in vitro* system It is possible that normal human lymphocytes can be transformed *in vivo* by EBV and grow progressively in the immunosuppressed heterologous hosts

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matters arising

Variation of *G*

DEARBORN and Schramm have considered limits on the variation of *G* imposed by the existence of clusters of galaxies and globular clusters of stars[1] Their argument is that if *G* has been varying too rapidly the clusters would have dispersed and we would not see them now They conclude that my theory of the variation of *G*, based on the Large Numbers Hypothesis, is untenable

These authors (and several other workers in the field) have misunderstood the main feature of my theory They work with a Newtonian theory in which gravitational mass can vary independently of inertial mass, so they are adopting a pre-Einstein view of gravitation On such a basis there would be no explanation for the motion of the perihelion of Mercury

The coefficient *G* has dimensions and its value depends on what units one uses One might refer it to standard units of physics, g, s and cm Then the question whether *G* varies depends on how these units are defined and is not a fundamental question of physics One should avoid man-made units and use only those provided by nature One may take units of time and distance provided by atomic clocks and the velocity of light, and a unit of mass provided by an atomic particle, say the proton Referred to such units the variation of *G* has an absolute meaning

If this *G* varies, the question arises of how to fit it into physical theory without destroying the successes of the Einstein theory A natural way of doing that is to suppose that all the laws of classical mechanics, including the Einstein theory, are applicable only when referred to suitable units that differ from the atomic units This is a development of an idea originally proposed by Milne[2], that there are two scales of time that are important in physics

Let us call the units that must be used for the laws of classical mechanics to apply 'mechanical units' For any problem involving dynamical motions and not referring to atomic processes one may work with mechanical units, and the calculations will then not be affected by the variation of *G* referred to atomic units This variation may be ascribed to the variation of the atomic units referred to the mechanical units Thus calculations of the stability of clusters

can throw no light on the variation of *G*

One can obtain evidence about the variation of *G* referred to atomic units by making astronomical observations with atomic apparatus One method is to observe accurately the times of lunar occultations with atomic clocks This method has been used by Van Flandern[3] who has obtained evidence that *G* does vary Another method is to work with I I Shapiro's observations of radar reflected by the planets These are not yet sufficiently accurate to give a definite result, but one can hope that they will be in the near future

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Future impact of fossil CO₂ on the sea

WHITFIELD[1] has challenged, on chemical grounds, the conclusion[2-4] that uptake of fossil CO_2 by the sea will eventually lead to unsaturation of calcium carbonate The optimistic conclusion that "serious environmental effects are not likely in the foreseeable future" is unwarranted for the following reasons (1) Whitfield's theoretical analysis obviously fails to maintain equivalence between anions and cations as CO_2 is taken up by seawater This impossible situation means only that the increase in total CO_2 predicted by mixing models is incompatible with the chemistry, changes in the degree of calcium carbonate supersaturation with increasing CO_2 partial pressure (P_{CO_2}) requires further analysis (2) Whitfield assumed too high an initial concentration of total inorganic carbon (ΣCO_2) in seawater, equivalent to $2\,66 \times 10^{-3}$ M We have measured ΣCO_2 in hundreds of samples of surface seawater (ref 5 and A W F, and J L E, unpublished observations) as have Li *et al*[6], which generally falls in the range $2\,05 \pm 0\,05 \times 10^{-3}$ M For an initial seawater ΣCO_2 of 2 05 (pH 8 00) and using MacIntyre's[7] values of the stoichiometric solubility product constants (K_{sp}') of calcite ($10^{-6\,17}$) and

aragonite ($10^{-5\,97}$), the carbonate concentrations for aragonite and calcite saturation are, respectively, $1\,07 \times 10^{-4}$ M and $0\,68 \times 10^{-4}$ M ($Ca^{2+} = 10^{-2}$ M), the corresponding reductions in carbonate are $0\,99 \times 10^{-4}$ M and $1\,38 \times 10^{-4}$ M, respectively The latter is only 60% of the value estimated by Whitfield for his higher ΣCO_2 for seawater (3) The degree of present-day supersaturation of seawater with respect to aragonite and calcite, and how this will change with increasing P_{CO_2}, depend on the choice of the various equilibrium constants of the ionic equilibria involved, about which there is some lack of concensus The degree of supersaturation is expressed by the ratio aCa_{2+} $a_{CO_3}{}^{2-}/K_{sp}$, where K_{sp} is the thermodynamic solubility product constant of $CaCO_3$, the *a*s are ionic activities γM, (where γ is the ion activity coefficient) Berner[8] measured γCa^{2+} to be around $0\,21 \pm 0\,01$, and $\gamma_{CO_3}{}^{2-}$ to be around $0\,022 \pm 0\,004$, depending on the chlorinity, in reasonably good agreement with Garrels and Thompson[9] The very low activity coefficient of $CO_3{}^{2-}$ ion reflects the fact that most of the carbonate ion in seawater is complexed with various cations Using K_{sp} values from Latimer[10] and Jamieson[11] (K_{sp} at 25° C = $10^{-8\,24}$ for calcite, $10^{-8\,15}$ for aragonite), Berner calculated the degree of supersaturation for a typical warm (25° C) surface seawater to be 2 8 for calcite and 1 8 for aragonite On the other hand Li *et al*[6] conclude on the basis of their measurements and MacIntyre's values of K_{sp}' that the degree of supersaturation of calcite and aragonite in seawater is about 5 5 for calcite and 3 5 for aragonite There is no question that surface seawater is supersaturated with respect to both minerals at the present time, but there is some uncertainty as to the degree (4) The solubility of CO_2 and $CaCO_3$ (both aragonite and calcite) in seawater increase with decrease of temperature Therefore one needs to be concerned not only with what may happen in warm, tropical seas (at about 25° C) but also the subtropical ocean which Whitfield did not consider At mid to high latitudes the average sea temperatures are much lower and these areas would be the first to achieve undersaturation of aragonite with increase of P_{CO_2} Fortunately, the majority of calcareous organisms which flourish in the cold, subtropical seas are calcitic, and therefore less soluble, but aragonitic forms do occur[12] We have

observed (our unpublished observations) that surface seawater equilibrated at 10 °C with CO_2 at $P_{CO_2} = 1,000$ p p m is unsaturated with respect to aragonite, aragonite dissolves under those conditions One may therefore ask whether an atmospheric concentration of CO_2 three times the present value is likely to result from future fossil CO_2 production Zimen and Altenhein[4] estimated that P_{CO_2} values of 1,000 p p m will occur by about the middle of the next century We believe an atmospheric level of 1,000 p p m will occur sooner as Zimen and Altenhein's estimate is based on a box model in which CO_2 uptake by the sea is not restricted by the chemistry Our experiments show that ΣCO_2 of seawater increases much more slowly than the box model allows and thus the sea will not absorb as much CO_2 as Zimen and Altenhein calculated Consequently, P_{CO_2} will increase faster than predicted by their model The ocean's ability to absorb the anticipated future fossil CO_2 emissions is so limited that it is not so much a question of whether a level of 1,000 p p m can be reached as to when it will be reached (6) Elsewhere, Whitfield[13] re-examined the problem of $CaCO_3$ unsaturation as a function of P_{CO_2} and temperature He concluded that aragonite unsaturation can be expected to occur in subtropical seas (average temperature about 10 °C) before the middle of the next century His theoretical result is in good agreement with our experimental observations We conclude therefore that there are grounds for serious concern about the impact of fossil CO_2 emissions of the ocean.

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Dr Whitfield replies — Fairhall and Erickson[1] have objected to several facets of a recent paper[2] in which I presented the chemical consequences of certain models[3-6] which have been used to pre-

dict the rate of fossil CO_2 accumulation in the oceanic mixed layer They emphasise[1], as I have done elsewhere[7,8], that the calculations[2] at 25 °C result in an increase in the carbonate alkalinity (CA) ($CA = [HCO_3^-]+2[CO_3^{2-}]$) which, as they conclude, indicates "that the increase in total CO_2 predicted by the mixing models (refs 3–6) is incompatible with the chemistry" The calculations[2,8] indeed provide strong grounds for rejecting the mixing models of Fairhall[5,6] and of Zimen and Altenhein[3,4] which predict gross accumulations of fossil CO_2 in the oceanic mixed layer I am glad that there is now some agreement on this point

The high value of ΣCO_2 that I used does not materially affect my conclusions Analyses of seawater from Plymouth Sound, using the titrimetric procedure described by Edmond[9], gave ΣCO_2 values that are comparable with those reported[1] (Table 1) The lower ΣCO_2 values, however, do not necessarily imply lower values of $[CO_3^{2-}]$ The carbonate alkalinity is a much better diagnostic criterion than ΣCO_2 as it also incorporates the effects of pH on the CO_2 system[7] It is possible to calculate the partial pressure of carbon dioxide required to reduce these samples to saturation level with respect to calcite or aragonite and also the pH at saturation level using the procedures described earlier (ref 2, equations (4), (6) and (7) and ref 7, equation (6)) MacIntyre's original value[10] ($10^{-6\ 26}$) was used for the solubility product of calcite at 25 °C and $35^0/_{00}$ salinity rather than the higher value ($10^{-6\ 17}$) quoted by Fairhall and Erickson[1] Similar values

have resulted from the recalculations of MacIntyre's data by Ben-Yaakov and Goldhaber[11] ($10^{-6\ 28}$) and by Ingle *et al*[12] ($10^{-6\ 30}$) The value quoted by Fairhall and Erickson[1] for the solubility product of aragonite is correspondingly higher than the original value

For Plymouth Sound samples at 25 °C, P_{CO_2} values seven to thirteen times those observed at present would be required for the mixed layer to approach the saturation level for calcite, depending on the solubility estimates used (Table 1)

These values are in excess of the most pessimistic predictions made so far for the accumulation of fossil CO_2 in the atmosphere[3,4] In some instances the calculated P_{CO_2} values approach the threshold limit value of toxicity (5×10^{-3} atm) They certainly identify as unrealistic those calculations[3,5] indicating that calcite saturation may be approached at 25° C following only a 50% increase in the current P_{CO_2} level The P_{CO_2} values for aragonite saturation at 25 °C are between five and eight times the present value These facts support my original conclusion[2] that, at 25 °C, "no account need be taken of the calcium carbonate equilibria since the mixed layer is supersaturated throughout and is likely to remain so" The editorial remarks appended to my original paper[2] which suggested that "serious environmental effects are not likely in the foreseeable future", do not, however, reflect my own conclusions

Preliminary experimental[1] (Table 1) and theoretical[7] results suggest that temperate waters (10 °C) may approach

Table 1 Conditions necessary to achieve saturation with respect to calcite and aragonite for samples taken from Plymouth Sound using different estimates of the mineral solubilities (salinity range 34 69‰ to 34 95‰)

Sample no	ΣCO_2 (meq l⁻¹)	CA (meq l⁻¹)	Mineral	Carbonate solubility ref	$-\Delta[CO_3^{2-}]$* (mmol l⁻¹×10)	pH s†	(P_{CO_2}) s† (atm×10³)‡
1‡	2 112	2 410	Calcite	2,14	2 72	7 12	4 41
				7,12	2 61	7 24	3 32
				10,13	2 52	7 33	2 68
			Aragonite	14	2 47	7 37	2 43
				7,12	2 43	7 40	2 26
				10,13	2 17	7 56	1 53
2‡	1 995	2 259	Calcite	2,14	2 38	7 15	3 88
				7,12	2 27	7 27	2 90
				10,13	2 18	7 36	2 33
			Aragonite	14	2 13	7 40	2 12
				7,12	2 09	7 43	1 97
				10,13	1 83	7 59	1 33
3§	2 112	2 282	Calcite	7,12	1 36	7,53	1 46
				10,13	1 19	7 67	1 04
			Aragonite	7,12	1 18	7 68	1 01
				10,13	0 84	7 87	0 63
4§	1 993	2 140	Calcite	7,12	1 13	7 56	1 28
				10,13	0 96	7 70	0 90
			Aragonite	7,12	0 95	7 71	0 88
				10,13	0 61	7 90	0 55

*Number of moles of carbonate per litre that must be removed to achieve saturation
†Subscript S applies to seawater at saturation with respect to the mineral shown if $[Ca^{2+}] = 10$ mmol l⁻¹
‡Sample adjusted to 25 °C
§Sample analysed at ambient temperature of 10 °C

saturation with respect to aragonice when P_{CO_2} attains approximately three times its present value The biological implications of such a change are unknown The attainment of the saturation level may in itself be less significant than the accompanying fall in the pH of the mixed layer

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Atmospheric halocarbons and stratospheric ozone

LOVELOCK[1] reports atmospheric analysis data for the fluorochlorocarbons (FCCs) CCl_2F_2 and CCl_3F in addition to the chlorocarbons (CCs) CCl_4, $CHCl_3$, CH_3Cl_3, $CHClCCl_2$, and CCl_2CCl_2 The synthetic FCCs are presently of concern as possible catalysts for the destruction of stratospheric ozone Although Lovelock states that "Molina and Rowland[2] rightly warn of a potential hazard to stratospheric ozone should the emission of the FCCs continue to grow unchecked", the levels of the CCs reported led him to conclude that "unless there is some special additional effect from the release of chlorine at higher altitudes, there are no grounds for singling out the FCCs as more hazardous than the other halocarbons which penetrate the tropopause"

I do not disagree with that as a statement of the present state of affairs, but I suggest that the conclusion, as stated, is likely to cause confusion as it does not distinguish between the present condition and the probable future values of the CC/CC ratio, the issue of central concern The following points seem to have been made by Lovelock or might be reasonably concluded from information presented by him (1) Present halocarbon levels are at the edge of significant participation in stratospheric ozone destruction cycles (2) CCl_4 and other CCs are present in far larger amounts than can be explained by reasonable estimates of synthetic sources, the present concentrations of CCs then represent essentially natural levels and are therefore not likely to change significantly in time

(3) The FCC analysis data suggest, however, that most of the FCC production to date is still present in the troposphere and lower stratosphere So the sinks for the naturally-occurring CCs do not seem to be significant for the FCCs For example, use of Lovelock's mole fraction of $1·0 \times 10^{-10}$ for CCl_2F_2 and an atmospheric scale height of 7 3 km lead to a value of $1·3 \times 10^9$ kg for the amount of this compound in a shell 10 km thick around the Earth This estimate is based on a low value for the tropospheric volume, neglects stratospheric content, and uses an analysis significantly lower than reported elsewhere[3] Use of a recent production rate of $0·5 \times 10^9$ kg yr^{-1} and exponential growth with a 3 5-yr doubling period observed back to 1960 (ref 4) gives an estimate of $2·5 \times 10^9$ kg for industrial production to date Thus a large fraction of the FCCs ever produced can still be accounted for in the atmosphere The only significant loss process would then seem to be diffusion past the tropopause and subsequent photolysis in the middle stratosphere

I conclude that the problem is not the consideration that the FCCs might have some special stratospheric reactivity in contrast to the effects of the CCs, as suggested by Lovelock, but rather that the FCC concentrations are likely to continue to grow to critical levels This has been illustrated by the model calculations of Cicerone, et al[4] Thus the FCC/CC ratio in the troposphere is most likely to increase steadily with time to a value significantly greater than unity with resulting future consequences for the ozone levels

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Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature* The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered

PROFESSOR LOVELOCK REPLIES—If the models of stratospheric ozone destruction by odd chlorine are assumed, then I agree with Chesick that the uninterrupted exponential growth of FCC emissions must eventually establish an atmospheric concentration hazardous on a global scale In this limited context the only point at issue is when this concentration will be reached The atmospheric significance of a compound depends on the rate at which it releases chlorine in the stratosphere and this is a function of residence time as well as of abundance The FCCs have residence times of more than 50 yr whereas the CCs other than CCl_4, have residence times of less than one year A large fraction of the FCCs entering the stratosphere are returned to the troposphere unchanged, but most of the CCs entering the stratosphere are destroyed there

I suggested that there might be a large natural chlorocarbon cycle[1] Evidence gathered during the past six months considerably strengthens this conclusion R A Rasmussen (personal communication) has found substantial concentrations of methyl chloride in rural air on the West Coast of the USA I have confirmed this finding for air coming from the Atlantic with concentrations of methyl chloride now exceeding 10^{-9} by volume As our investigations proceed, the sum total of atmospheric chlorine carriers discovered grows much more rapidly than does the release of FCCs The measured ratio of CC to FCC chlorine is now approaching four

The models of ozone destruction by odd chlorine are plausible and do give cause for concern but in the debate it tends to be forgotten that the models are entirely unconfirmed by direct observations in the stratosphere

When all these factors are taken into account it does seem that there is time to make those stratospheric measurements from which a realistic upper limit to the concentration of anthropogenic chlorine compounds can be decided Tropospheric measurements are also needed to complete our understanding of the natural chlorine cycle

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Alteration of chicken melanocytes by DNA

LANZA has reported that injection of Harco chicken DNA into 5-d-old White Plymouth Rock embryos permits production of melanin beyond day 14 of incubation, in contrast to uninjected embryos[1] He cites Hamilton's work, in which melanocytes were observed in embryos of all fowl examined, including breeds with white plumage, the melanocytes of white breeds were initially capable of melanin synthesis but degenerated

before hatching[2] Thus, Lanza states that DNA treatment extends the life span of such melanocytes Because of related studies in my laboratory, I would like to offer two possible interpretations of Lanza's observations

We have reported that injection of chicken DNA into White Leghorn eggs induces two types of effects on the embryos, depending on incubation conditions a lethal effect which may be mediated by a genetic-like mechanism and a survival-enhancing effect involving a non-genetic mechanism[3] Both effects are inducible by isologous DNA Highly polymerised DNA can also enhance mammalian cell viability *in vitro* under conditions excluding a genetic-like mechanism[4] Such a phenomenon might account for the extended survival of melanocytes in Lanza's system While we did not observe induction of pigment in more than 200 hatched chickens whose eggs had been injected with non-degraded DNA (either isologous or Rhode Island Red), our experimental procedures differed extensively from Lanza's

Second, several groups, including ours, have reported induction of melanin in mammalian cells by exogenous DNA[5-7] Detailed analysis of our data indicated that our effect was the result of alteration of a regulatory gene instead of a structural gene[8 9] This would seem to be the case for Lanza's system if it involved genetic transformation for pigmentation (instead of survival) As noted above, his target cells presumably contained genetic information for melanin production before DNA treatment

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DR LANZA REPLIES—The points made by Dr Glick are well taken A survival-enhancing effect similar to that cited[3], could indeed have extended the life span of the melanocytes reported

I would like to point out that all five of the embryos which had patches of pigmented feathers were examined between days 14 and 16 of incubation Although one of the newborn chicks developed some pigmented feathers, no pigment was observed in the juvenile down of any chickens hatched in earlier experiments This suggests that all the melanocytes were not altered permanently, and those that were, were too few in most cases to synthesise enough melanin to be detected in the juvenile down of a newborn chicken

Glick's experimental procedures differ extensively from the experiment reported Alterations in the embryonic stage would have gone undetected because he observed only hatched chickens It should be emphasised that the pigmentary genes in the White Leghorns referred to in Glick's response are considered dominant, whereas those in White Plymouth Rocks are considered recessive[10] I am led to conclude that any genetic transformation would be non-pigmentary and would directly involve the genes responsible for the degeneration of the melanocytes

Although Glick's analysis of induced melanin synthesis in mammalian cells indicated that the effect was due to a regulatory gene instead of a structural gene[8], he should not preclude the possibility of a structural gene defect in White Plymouth Rock melanocytes The lack of pigmentation in most mammalian cells results from a gene–enzyme defect, in which tyrosinase fails to catalyse the reaction in which tyrosine is hydroxylated to dihydroxyphenylalanine This is not the case, however, with White Plymouth Rocks as Glick stated, the melanocytes in that system were initially capable of melanin synthesis Therefore genetic transformations involving pigmentation in this breed, would not necessarily be the result of a regulatory gene

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Schwarzschild orbital topography and high Doppler blueshifts

CHITRE, NARLIKAR AND KAPOOR[1] discuss the high Doppler blueshift of forward light emission from material particles in circular orbit in a Schwarzschild field at and near $r = 3GM/c^2$, which they claim is the radius of the unstable circular orbit (meaning, apparently, "the marginally stable circular orbit")

This supposed effect rests on a basic confusion concerning the orbital topography and energetics in the Schwarzschild field Outside the event horizon, at $r = 2GM/c^2$, there are three important circular orbits[2] the marginally stable circular orbit at $r_{ms} = 6GM/c^2$, inside which there can be no stable circular orbits (Chitre *et al*[1] mistakenly put this at $r = 3GM/c^2$), the marginally bound circular orbit at $r_{mb} = 4GM/c^2$, inside which there can be no bound circular orbits, and the photon orbit at $r_{ph} = 3GM/c^2$, which has an infinite energy

per unit mass and requires a tangential velocity $v_{tang} = c$ Chitre *et al*[1] seem to have confused the marginally stable circular orbit with the photon orbit So it is not surprising that they obtain an infinite blueshift there, since any particle in such an orbit must have $v_{tang} = c$ But, of course, massive particles are barred from such orbits, and those in circular orbits of radii approaching $r = 3GM/c^2$ are not only in an unstable configuration but also highly unbound ($E \gg 1 mc^2$) In any realistic physical situation they will all move along outward spiral orbits

Near $r_{ms} = 6GM/c^2$ particles will make many revolutions, but there will be no net Doppler blueshift, since for these $v_{tang} \simeq c/2$ As particles reach $r = r_m$ through, for example, viscous drift, the plunge towards the black hole along spiral orbits For a test particle moving along such a spiral geodesic, conserving total energy ($E_{ms} = 0 943\ mc^2$) a angular momentum ($L_{ms} = 3 46GMm/$ where m is the mass of the test particle 4 85 revolutions will be executed between $r = r_{ms}$ and $r = 2GM/c^2$ (W R unpublished), and the first four revolutions will be between $r = r_{ms}$ a $r = 5GM/c^2$ If dissipative and radiative processes are important, the spiral orbit will have even fewer revolutions S there seems to be little reason to expect that there could be a thin disk of massive particles executing even approximate circular orbits in the "high blueshi region" near $r = 3GM/c^2$

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DRS CHITRE, NARLIKAR AND KAPOO REPLY—We meant what we said in ou paper The orbit in question is close t $r = 3GM/c^2$ as stated, and is not nea to $r = 6GM/c^2$ as Stoeger would have u suppose We are aware that these orbit are unstable and it is not intended tha the particle should circulate in suc orbits for ever The circular orbit meant to be an approximation to th geodesic trajectory of the infalling pa ticle with high energy and small impac parameter, as discussed by Misner *et al* Those authors have discussed gravitation synchrotron radiation from particle transiting close to $r = 3GM/c^2$ The and related references have already bee cited in our paper so no further clari cation is necessary

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The reward system needs overhauling

IN this last week, and in this International Women's Year, the Royal Society marked the occasion by electing a woman as one of the thirty-two new fellows, and Fred Hoyle put the cat among the pigeons in Nobel wonderland by saying that Jocelyn Bell (now Jocelyn Burnell) ought to have got the prize for the discovery of pulsars One can always rely on the reward system in science to breed controversy

On the making of Fellows of the Royal Society there is, of course, no end of folk-lore, gossip and scandal This has been true since at least the eighteenth century, when membership of the society was a fashionable thing for London gentlemen As late as 1830 Charles Babbage could write "several times, whilst I have been consulting books or papers at Somerset House, persons have called to ask the Assistant-secretary the mode of becoming a member of the Royal Society I should conjecture, from some of these applications, that it is not very unusual for gentlemen in the country to order their agents in London to take measures for putting them up at the Royal Society" Now the society is so purged of its non-scientific attachments that it can reasonably be accused of being too addicted to the rewarding of those who practise science and too little aware of those who teach, communicate, document, administrate and philosophise about science It is somewhat silly that a first-class administrator with major achievements in advancing the organisation of British science should have to be admitted this year on the basis of being distinguished for scientific work which he had to give up ten years ago

It would be invidious to name individuals whose distinction will never be rewarded under the present state of affairs (*Nature* keeps a list of fifteen or so) But it should be pointed out that the mechanism for recognising "conspicuous service to the cause of science" exists but is restricted to one extra fellow a year—at present only eleven fellows are in on that ticket, including two royals and five politicians The mechanism also exists for "general candidates" to be admitted, although the committee charged with presenting names does not seem to have outstanding success in leavening the fellowship By all accounts the process of selecting fellows is a parochial affair in which no external advice is sought, this may assure main-line excellence, but it doesn't recognise the new dimensions of science which represent its internal and external interactions

In the past 30 years the representation of women in the society has actually declined Of those present fellows, seven were elected between 1946 and 1955, eight between 1956 and 1965 and eight in the past 10 years In the same period the total number of new fellows elected has risen from 25 to 32 annually The society can hardly be blamed for the shortcomings of a system in which it is still difficult for women in large numbers to pursue science with anything approaching the single-mindedness that intellectual distinction demands On the other hand women, with every justification, are looking for some redressing of the balance This has called in some countries for positive action by institutions, and it would be good to see the Royal Society give a lead in asking the question—how can we recognise the contribution that women make to science? More than 10% of the authors of papers in this journal are women and most of our authors are relatively young, yet by the time that they have reached an age at which they are recognised as distinguished, the proportion has dropped to about 3%

Finally, astronomy has now had the Nobel plague descend on it The joy of astronomers that at long last a Nobel prize is within their grasp is likely to be shortlived when they discover how divisive the whole concept of vast cash awards and hob-nobbing with royalty is to the scientific community Fred Hoyle's complaint, however justified or unjustified, is only an early shot in a battle which will set scientists against each other in years to come as more astronomers get rewarded, and others go unrewarded

The trouble with Nobel prizes is threefold First, there are clearly not enough to go around, and hence there is bound to be inequity in their distribution Second, they tend to be confined to a restricted number of subjects, giving a false impression of the relative importance of various fields Third, they confer an unreasonable amount of credit on their recipients leading to Merton's well-documented Matthew effect ('to him that hath, it shall be given') and often to the less well-documented Boeing complex ('given the option between jetting around the world lecturing on things you don't know much about and staying at home working, go for the jet-trip any day')

In certain subjects and certain laboratories one gets the distinct impression that the pursuit of Nobel prizes is the ultimate aim, rarely spoken of but rarely very far below the surface Ambition is a fine thing but these inequitable, divisive, flattering prizes generate obsession Isn't it time they were abolished? □

Plutonium particles: some like them hot

The Medical Research Council's 1975 report The Toxicity of Plutonium (Nature, 253, 385) concludes that "there is no evidence that irradiation by 'hot particles' in the lung is markedly more hazardous than the same activity uniformly distributed or that the currently recommended standards for inhalation of plutonium are seriously in error" Report R29 (1974) of the National Radiological Protection Board (NRPB) states that "there is no biological evidence available at present which suggests that 'hot spots' carry a higher risk of cancer induction" But in the March 20, 1975 New Scientist, Dr A R Tamplin warns "The plutonium exposure standards must and will eventually be made more restrictive by a factor approaching 1,000" Behind this disagreement is a major controversy over the risk of lung cancer from inhaled insoluble particles of intensely radioactive alpha emitters such as plutonium In that controversy—unresolved by any direct data—lies a source of uncertainty about the future of nuclear power A report by Amory B Lovins and Walter C Patterson of Friends of the Earth

SINCE the late 1960s, many experts— Geesaman, Langham, Long, Morgan among others—have expressed concern about the formidable but highly uncertain toxicity of plutonium (Pu) aerosols On February 14, 1974, Drs Tamplin and Cochran of the Natural Resources Defense Council (NRDC) took action They petitioned the United States authorities to reduce the maximum permissible lung burden (MPLB) and maximum permissible concentration in air (MPC$_a$) of insoluble Pu by 115,000 times Also in 1974, the US Atomic Energy Commission published two defences of present standards, LA-5482-PR and WASH-1320, and in May and November NRDC published interstitial rebuttals to which the AEC declined to respond in writing or otherwise The NRDC petition is under review by many US official bodies, and the AEC's successor agency should announce its next move in April

The cancer risk from microscopic inhaled particles of insoluble Pu and similar actinides arises because such particles can deposit in deep lung tissue, can stay there for a year or more, and, being hard alpha emitters, can irradiate only that tissue which is within ~ 40–45 µm This last property makes it hard to determine the MPLB and MPC$_a$, for these are derived from an international standard which says merely that lungs should not be exposed to more than 15 rem of radiation per year Fifteen rem is equivalent to 1 5 rad of alpha radiation (since alpha radiation is considered 10 times as carcinogenic as gamma radiation), and a rad, the basic unit of "radiation absorbed dose", is that amount of radiation which deposits 100 erg of energy per gram of tissue irradiated But which tissue is that? Present practice is to average the alpha dose over the entire 1000-g pair of lungs whether it all receives alpha radiation or not

This practice is illustrated in Table 1, which shows approximate data for some hypothetical distributions of one MPLB of the dominant isotope ^{239}Pu (16 nCi = 0 016 microcurie, the amount whose

annual radiation dose, averaged over 1,000 g of lung tissue, would be 15 rem) For convenience, and with no pretence at realism, Table 1 assumes that this MPLB is uniformly distributed in the lungs with varying degrees of aggregation, ranging from a single 37-µm particle (actually too big to be respirable) to ~ 50 million 0 1-µm particles But each ^{239}PuO$_2$ particle, however large, can only irradiate ~ 50–100 of the several hundred million alveoli, therefore an MPLB uniformly distributed as particles larger than ~ 0 15 µm must irradiate some lung tissue at more than 15 rem per year and some other tissue not at all (Non-uniformly distributed particles, as they would be in reality, would give non-uniform doses at even smaller particle sizes) Nonetheless, the mean lung dose, now used to set the MPLB and MPC$_a$, would be 15 rem per year in each case shown, and each would be assumed to carry the same cancer risk

The issue posed by Table 1 is whether intense irradiation of a very small mass of tissue is more carcinogenic than less intense irradiation of a proportionately larger mass, that is, if a given amount of intense irradiation is distributed more evenly over lung tissue, whether the greater number of cells at risk exactly compensates for the smaller risk per cell An hypothesis that this were untrue would imply that carcinogenesis is not linearly proportional to dose at very high dose levels because intense local irradiation can bring to the fore some new mechanism(s) absent or unimportant at lower doses (Loosely speaking, this is a "threshold" theory, but one applying to high doses, not low ones people who argue for non-linearity in the low-level radiation controversy tend to argue against it in the hot-particle controversy)

Such a high-dose mechanism might involve (for example) carcinogenic or contributory chemical activity by fragments of killed, fibrotic, liquefied, or otherwise seriously disrupted cells, rather than (or in addition to) "primary" or direct carcinogenesis by disruption of, say, a chromosome Such a "secondary" mechanism may coexist with other mechanisms, is plausible, and has respected adherents, but has not been directly demonstrated Neither, however, has any other proposed mechanism of radiation carcinogenesis, primary or secondary

The International Commission on Radiological Protection (ICRP) have sometimes had to introduce a Distribution Factor into the rad-to-rem conversion to allow for inhomogeneous doses within a "critical organ" Thus the dose limit for bone is based on uniformly deposited ^{226}Ra, but Pu, collecting at the bone surface, is considered ~ 5 times as radiotoxic, so a Distribution Factor of 5 is used to compute Pu bone dose Likewise, IAEA TR-142 (1973) reports

Table 1 Some illustrative and hypothetical uniform distributions of 16 nCi (= 1 MPLB) of ^{239}PuO$_2$ in 1 kg of lung tissue
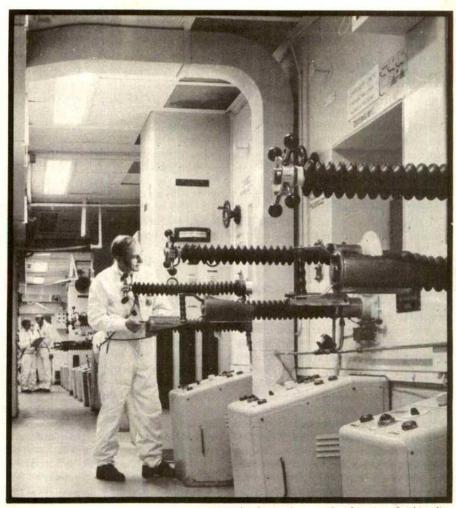
Number of particles	1	50	5×10^4	$1 5 \times 10^7$	5×10^7
Particle diameter (µm)	37	10	1	0 15	0 1
Particle mass (ng)	300	6	0 006	2×10^{-5}	6×10^{-6}
Particle activity (pCi)	16,000	300	0 3	0 001	3×10^{-4}
Notional lung mass irradiated	65 µg	3 3 mg	3 3 g	1 kg	1 kg
Fraction of 1 kg	7×10^{-8}	$3 3 \times 10^{-6}$	0 0033	1 0	1 0
Local tissue dose	200 Mrem/yr	4 Mrem/yr	4 krem/yr	15 rem/yr	15 rem/yr
Mean dose to 1 kg lung tissue	15 rem/yr	15 rem/yr	15 rem/yr	15 rem/yr	15 rem/yr

The distributions shown in the last two columns give a local-tissue dose equal to the average lung dose because the radiation fields of that many particles must overlap, whereas in the first three columns, the radiation fields from so few uniformly distributed particles are insufficient to "fill" the lung In reality, the distribution would not be uniform, and the latter position would arise with particles smaller than shown in the last two columns Two further points about half the energy of the alpha radiation is dissipated in the first 20 µm of tissue radius, or in about 1/8 of the volume irradiated by each particle, and since some attenuation of the alpha radiation in a spongy lung occurs in air rather than in tissue, the mass of tissue irradiated by each particle, and hence the reciprocal of dose to that mass, would be less in compact soft tissue such as skin

that "because it had been recognised that the dose distribution [from inhaled radon daughters] in the respiratory tract was inhomogeneous and the maximum alpha dose was delivered to the bronchial epithelium" rather than to the whole lung, the ICRP suggested a Distribution Factor of 10, owing to evidence that uranium miners were getting lung cancer more often than the average-lung-dose model had predicted. So far, no Distribution Factor > 1 has been applied to Pu in lung; and NRDC suggest, in effect, that the "critical organ" limited to 15 rem per year should be not the complete pair of lungs, but rather the tissue irradiated within it. Otherwise, using the dose-averaging concept, a Pu particle in beagle lung would be 8 times as carcinogenic as the same particle in human lung (neglecting species differences) simply because human lung is 8 times as large. Indeed, if each of two men had an identical Pu particle in his left lung but the right lung of one man had previously been removed, his computed risk of lung cancer from the particle would be twice that of the other man! This *reductio ad absurdum* shows the artificiality of averaging very localised doses over entire organs.

The ICRP have explicitly declined to offer guidance in this area: as the NRPB state, "radiological protection standards cannot be applied to small volumes as they have been derived from observation of biological effects in the whole body or in body organs." Experiments have shown, however, that intense irradiation of, or other insults to, small amounts of various tissues (including bronchial and lung tissue) can produce tumours or other lesions showing cellular changes characteristic of precancerous lesions. Indeed, Pu particles in rat lung have been shown to cause lesions similar to one observed around a 5-nCi [239]Pu particle excised in 1962 from a worker's palm: this lesion showed "severe" changes similar "to known precancerous epidermal cytological changes". There is strong evidence, too, associating a fatal soft-tissue sarcoma in a US worker with entry, through some minute cut, of Pu solution that had spilled on his hand. Thus cancer, or lesions suggestive of eventual cancer, is demonstrably induced in both man and laboratory animals by very intense irradiation of very small amounts of various tissues, including rat lung. But does the incidence of such effects exceed that observed for lower intensities applied to proportionately larger amounts of tissue?

No direct answer is available for inhaled insoluble Pu. Disquieting long-term experiments with beagles have shown that almost 100% of dogs inhaling Pu doses down to 200 nCi ($\sim 10^7$ particles) get lung cancer within $\sim$ 11 years, but experiments with lower doses will not yield data for many years. The evidence

Windscale: workers on the plutonium finishing line

must therefore be indirect, so one must decide what evidence is relevant and hence exactly what is a "hot particle". The February 1974 NRDC definition, based largely on work by Geesaman, is an alpha–emitting "particle in the deep respiratory tissue of such activity as to expose the surrounding lung tissue to a dose of *at least* 1,000 rem in 1 year." New data (including human autopsy data) discussed by NRDC in a February 1975 paper suggest that a lower limit for hot-particle activity might be anywhere from 0.07 pCi (derived from the 1 krem per year criterion) to perhaps 4.2 pCi, —a four-fold range of particle diameter— depending on the data and degree of conservatism chosen. NRDC also prefer to define the limit not by calculated local dose but by particle activity, which can be correlated with observable phenomena: some levels of activity produce fibrotic lesions whilst others do not.

Clearly it is necessary to assume some lower activity limit distinguishing the hot-particle carcinogenic mechanism from that of uniform dose, and any such postulated limit must have a certain arbitrariness which invites challenge. (The limit may not in reality be so clearly defined.) Suggestively, the 0.07-pCi limiting activity originally proposed by NRDC is substantially above the level at which

uniformly distributed particles totalling 1 MPLB cannot irradiate the whole lung (Table 1).

No quibbling can arise over whether a particle is a hot particle if its activity is orders of magnitude too low to cause observable discrete lesions. The particles used in many experiments, those produced by fallout from nuclear testing, and those inhaled by the often-cited 25 Manhattan Project workers are all in this category and hence cannot be used to test the NRDC hypothesis. At least 25 other workers did inhale hot particles from the 1965 Rocky Flats fire, and linear extrapolation from the beagle data suggests that these men are likely to develop lung cancer after an unknown induction period, probably of several decades.

Many critics of the hot-particle hypothesis do not state whether the experiments they cite involve hot particles. For example, the NRPB's dismissive review of the NRDC hypothesis relies heavily on an unpublished paper of Lafuma, comparing the incidence of lung cancer in rats after inhalation of "particulate" [239]Pu or "diffuse" [242]Cm. NRPB claim a lower incidence for the former doses, but give no data, and one is entitled to wonder whether the [239]Pu was in the form of hot particles, what the doses were, whether the lifespan of the rats exceeded the

induction time of the effects sought, what allowance was made for the 23-week half-life of ^{242}Cm and so on. Inquiries to NRPB in January, 1975 elicited the astonishing reply that such basic data as particle size and activity were not in Lafuma's paper and must be sought from him. (An immediate letter to that end has had no reply at this writing.) So it is not clear whether the NRPB's crucial experiments involve hot particles at all.

Even if they do, the data are still consistent with the hot-particle hypothesis. Experiments suggesting that tumour incidence per nCi is greater with uniform than with discrete dose distribution—or the opposite, as both can be shown—are not relevant to, nor a test of, the hot-particle hypothesis: the latter bears on the risk per hot particle, not the risk per nCi. If the hypothesis is right, then increasing the activity of a hot particle—one that is already large enough to bring into play the postulated high-dose mechanism of carcinogenesis—only further disrupts already-killed cells and does not significantly increase the risk from that particle, though it reduces the apparent risk per nCi. Thus the hypothesis already contains the concept of "overkill" or "wasted radiation". Table 2 shows six hypothetical experiments devised by NRDC: *A-D* suggest that discrete dose is less hazardous than uniform dose, *E-F* suggest the opposite, and *A-F* are all consistent with the hot-particle hypothesis.

The NRDC rebuttal of WASH-1320 appears to show that all experiments there cited are either irrelevant to the hot-particle hypothesis or consistent with it. The irrelevance arises because for example, experiments do not involve insoluble hot particles in lung, or use short-half-life actinides, or show rapid lung clearance, or have been interpreted on a risk per nCi basis, or use animals that do not survive the induction period, or do not wait for it to elapse, or mask the lung-cancer response with other pathological conditions, or do not involve significant samples.

The MRC report, apparently initiated by the NRDC petition, cites it as the work of "an organisation called the Environmental Defense Research Council concerned with problems of pollution in general" (wrong on both counts) and, like the NRPB report, ignores the two 1974 NRDC rebuttals which follow up the original petition and anticipate the MRC and NRPB arguments. (The MRC report does not even cite the two AEC responses to the NRDC petition.) By failing to read these papers or to contact NRDC, the MRC and NRPB have lost an opportunity to raise arguments not already canvassed. They have stressed the quantitative uncertainties (limiting particle activity and risk per particle) but have not disproved the basic thesis. Instead, they prefer to argue that "there is no evidence" for the NRDC hypothesis—

whilst ignoring much of the evidence NRDC cite in their original paper, notably reports of Pu-induced lesions in both rats and man. But the "no evidence" argument cuts both ways. There has been no concerted effort in Britain to imitate the new US Transuranium Registry of radiation workers, which it is hoped will eventually find the epidemiological evidence if it exists. To argue from the general absence of evidence (for which one has not looked) that there is no evidence for one's opponent's point of view is to write with an economy of truth.

The main drawback of the hot-particle hypothesis seems to be that it requires one to assume an unproven and somewhat vague cellular mechanism of radiation carcinogenesis operating at high dose levels. Yet critics of the hypothesis have been unable to demonstrate any mechanism underlying their own theories, and until they have done so, it is hard to see how other mechanisms can be definitely excluded. The resulting scientific uncertainty, and its likely persistence, raise an important ethical and political issue. We have an hypothesis, neither proved nor disproved, implying that the MPLB and MPC$_a$ for insoluble aerosols of Pu and similar actinides—inherent in a large-scale fission economy—should be reduced by orders of magnitude. We have two choices: (1) ignore the hypothesis as unproven, continue our research, continue to expose some radiation workers to hot particles, assume that these are innocuous, and proceed to make the world's energy supplies dependent on annual flows of hundreds of tons of Pu; or (2) provisionally adopt the hypothesis until it is disproven, tighten standards accordingly, and thus act with the caution, prudence, and conservatism which—we are told—are the basic principles of radiation protection. So far, regulatory bodies have taken the first course, gambling that exposed Pu workers will not begin, a decade or two hence, to show excess lung cancers as some analysts claim they are now beginning to show excess leukaemias.

The reason for this gamble may be related to a little-known fact: that even the present MPLB and MPC$_a$ for insoluble Pu are near the limits of practical detectability, not because we lack sensitive instruments but for fundamental statistical reasons. It is not clear that present monitoring methods at, say, Windscale can demonstrate compliance even with present standards: a "puff" release from,

say, a defective glove-box may be hidden in the 24-hour integration period of air samplers, and even a real-time radiation monitor could probably not detect significant Pu levels in a typical room before most of the air in it had been inhaled or changed. If the standards became much stricter, compliance could never be shown and the standards would have no operational meaning. Whether a Pu-fuelled industry could then operate would depend on whether the burden were on it to prove compliance (impossible) or on others to prove non-compliance (presumptive but debatable) and on how strictly the relevant duties were construed.

The questions of monitoring and enforcement are not academic; they are the crux of the matter, for a few decades from now the growing gap of magnitudes between world Pu stocks (thousands of tons) and the amount which can produce a nuclear explosion (a few kg) or give a beagle lung cancer (a few μg) will require impressively and increasingly diligent care to prevent releases through accident or malice. Pu metal, nitride and carbide can be pyrophoric, burning to respirable PuO$_2$; this insoluble ceramic is itself present in advanced nuclear fuel cycles, can pass in respirable sizes through high-efficiency filters, and has contaminated dozens of Pu workers in accidents here and abroad. Pu fires have released tens or hundreds of grams of PuO$_2$ from US facilities and far more within them. Pu could be released by fast-reactor accidents, weapons explosions, deliberate dispersal, or chemical or microbial action on Pu-bearing wastes (like the dilute alpha wastes buried at Drigg near Windscale). Biological pathways for reconcentration of Pu—now discharged in hundreds of Ci per year from Windscale into the Irish Sea—may exceed those now known, as Pu chemistry is very complex. Since the half-life of ^{239}Pu is 24,390 years, and since fallen-out aerosols may become resuspended (data on this vary over 11 orders of magnitude), dispersal may be essentially permanent. In an energy economy based on hundreds, then thousands, of fast breeder reactors, each containing several tons of Pu, even if Pu escape were controlled with the most exquisite care, the unavoidable limits of *in vivo* and environmental measurement would make it impossible to tell whether we were meeting stricter Pu standards or not. We could only tell from increased lung cancers—decades after the irrecoverable releases had occurred. ☐

Table 2 Some hypothetical hot-particle experiments

Experiment	A	B	C	D	E	F
Number of hot particles	6000	4000	2000	200	6000	4000
Total activity (nCi)	12	12	12	12	12	6
pCi/particle	2	3	6	60	2	1.5
Number of tumours observed	3	2	1	0	3	2
Tumours/nCi	0.25	0.17	0.08	0.00	0.25	0.33
Tumours/particle	0.0005	0.0005	0.0005	—	0.0005	0.0005

international news

'Jennifer' subterfuge scuppered

by Colin Norman, Washington

Hughes: obsessively secretive

THE dramatic revelation that the Central Intelligence Agency (CIA) last year secretly salvaged part of a Soviet missile-carrying submarine from the bottom of the Pacific, could hold some important implications for oceanographic research. Though such concerns are dwarfed by the wider political ramifications of the affair, some knowledgeable observers last week pointed out that the CIA's salvage job could affect the outcome of the Law of the Sea Conference which is now grappling with, among other things, issues concerned with freedom to carry out research in, on and under the high seas. Moreover, the affair could upset the flourishing co-operation between the United States and the Soviet Union on oceanographic research.
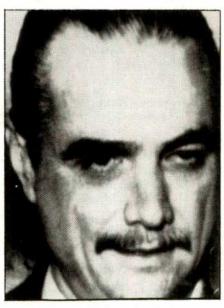
The operation had all the essential ingredients of a James Bond thriller, involving as it did a national intelligence operation, an elusive billionaire, an elaborate cover, and hundreds of millions of dollars. For five years it was also one of the Nixon and Ford Administration's best kept secrets, until a number of newspapers last week decided to print the details, in spite of frantic attempts by the CIA to keep it bottled up.

Known as Project Jennifer, the operation began to take shape soon after the submarine sank in about 16,000 feet of water in 1968, about 750 miles north-west of Hawaii. Intelligence sources in the United States knew the exact location of the vessel because they were able to pick up underwater explosions from it, but Soviet ships looked for it in the wrong area according to a report in the *New York Times*. When the Soviet ships abandoned their search, the CIA decided to try a salvage job itself.

The cover for Project Jennifer was inspired, to say the least. A special salvage ship was constructed for the CIA by Summa Corporation—a huge industrial concern operated and owned by Howard Hughes, the eccentric and elusive billionaire—and it was publicly billed as being the biggest seabed mining vessel ever constructed. Another essential component of the operation, a massive submersible barge, was built by the Lockheed Coporation; it was also depicted as playing a central role in bringing manganese nodules to the surface, but its sole function was to hide the submarine when it was recovered.

Because the nascent technology of seabed mining was jealously guarded in the early 1970s by the few companies hoping to scoop manganese nodules from the ocean floor, and because Hughes has a reputation for obsessive secrecy, few questions were raised about the clandestine nature of the operation. Moreover, the submarine went down in an area of the Pacific where manganese nodules are known to litter the seabed.

The salvage ship, known as the Hughes Glomar Explorer, was launched in 1973, and it made its first salvage attempt in July and August last year. According to reports, it succeeded in lifting the submarine from the sea bed, but recovered only a part of it. Although some of the accounts are conflicting, it is widely believed that the CIA failed to retrieve the two chief items it wanted—the nuclear-tipped missiles and code machines — and another attempt was being planned for later this year. But publication of the details has, apparently, scuppered that plan.

As far as the implications for research are concerned, one important aspect of the affair is that the Glomar Explorer was operated by Global Marine Inc., a company which has built, owns and operates a number of oceanographic research vessels. Prominent among its fleet is the Glomar Challenger, the highly successful drilling vessel which is operated under contract to the National Science Foundation, and which has been conducting the six-year-old Deep Sea Drilling Project (DSDP).

Last year, the Soviet Union joined the DSDP, and it is now contributing $1 million a year to the project. Although the Soviet government had made no comment on the CIA's salvage job by the end of last week, one US official connected with the DSDP speculated privately that the Soviet Union may now reconsider its participation, in view of Global Marine's connection with the CIA.

Moreover, one newspaper account even suggested that the Glomar Challenger had played a direct role in the initial reconnaissance for Project Jennifer, but that report was hotly denied by the National Science Foundation (NSF). For one thing, as the vessel's publicly available logs show, it was not in the vicinity of the submarine at the time, and for another, two Soviet Academicians were aboard when it was alleged to be scouting out the wreck. An NSF spokesman also pointed out that the Glomar Challenger has been operated continuously under contract to the foundation since it was launched in 1968, and at no time could it have been diverted to the CIA's operation.

As for the Law of the Sea Conference, one issue to be resolved is the extent to which oceanographic research should be allowed to take place unhampered both on the high seas and also within the 200-mile 'economic zones' which may be established around the coasts of maritime states. The nub of the issue is that most industrialised nations are arguing for scientific research to be permitted unimpeded outside territorial waters. But the developing nations have generally argued that research operations could provide a cover for economic exploitation of seabed minerals, and for espionage. The CIA's cover for Project Jennifer provides all the justification in the world for such fears.

The point has not been lost on delegates attending the Law of the Sea

Conference which, ironically, reconvened last week in Geneva. According to another *New York Times* account, Christopher W. Pinto, a delegate from Sri Lanka and a leading spokesman for the developing nations, has already said that the CIA operation has undercut the argument for freedom for research. He is quoted as saying that the developing nations have been suspicious that research activities may provide a cover for other operations, and "now that this is confirmed, they [the developing countries] can be more forceful".

Another aspect of the salvage operation is important for the development of undersea technology: by depicting the Glomar Explorer as a deepsea mining vessel, the CIA provided the greatest possible incentive for other mining companies to step up their operations. When the ship was being built and operated, it was generally assumed that Hughes was leading the field in the race to scoop manganese nodules from the deep sea bed.

During Congressional hearings in 1973 and 1974, for example, representatives from other prospective seabed mining companies argued vigorously for Congress to pass legislation which would offer them some financial security so that they could raise and invest vast amounts of capital in the technology. Although Congress did not pass the legislation, there is little doubt that the prospect of Hughes beating the rest of the industry to the seabed riches stimulated frenzied efforts in other companies.

It is unclear, however, whether or not the Glomar Explorer could be used for seabed mining. It is also unclear who would own and operate it for such activities, although a good case could be made out for the argument that it belongs to the federal government.

As a footnote to the operation, it should be pointed out that Project Jennifer reportedly cost about $350 million, which is considerably more than the federal government's entire budget for oceanography for the past five years. The money was, however, provided with the knowledge of very few Members of Congress and of only a few people in the Administration. ☐

Call for cuts in astronomers

by Colin Norman, Washington

A COMMITTEE of the National Academy of Sciences this week made the painful and perhaps unprecedented recommendation that graduate departments in the United States should reduce their output of astronomers because of dismal employment prospects and sagging financial support for astronomy.

Although other groups of scientists have urged that production of PhDs in some fields should level off, few have openely called for graduate education to be cut back. But the situation in astronomy is considered so serious that "we can't just sit and wail about it", Dr Leo Goldberg, Director of the Kitt Peak Observatory and chairman of the Academy committee, said in an interview last week. He added that even if enrolment in PhD courses in astronomy and astrophysics were cut in half, there would still be too many astronomers chasing too few jobs.

The problem is familiar enough. Force-fed by direct government support and by the burgeoning space programme in the early 1960s, astronomy experienced a period of spectacular growth, and graduate departments expanded rapidly. But, just as large numbers of astronomers began to emerge from the universities with freshly minted PhDs in the late 1960s

and early 1970s, government support slackened and job opportunities rapidly dried up. And the situation is getting worse because, unlike most other fields of science, graduate enrolment in astronomy is continuing to increase so that the supply and demand for young astronomers is going to get further and further out of balance.

To put the matter in perspective, Goldberg suggested that employment is likely to be available for only about 50 new astronomers a year, but last year alone the universities awarded more than 180 PhDs in astronomy and astrophysics. Adding to the problem is the fact that many people with doctorates in other branches of physics will be chasing jobs in astronomy and moreover, there are now between 150 and 200 astronomers holding temporary postdoctoral appointments who have no prospects of moving on to full-time employment, Goldberg said.

The growth in university astronomy departments may reflect the fact that the science continues to be intellectually exciting. But these days, intellectual excitement does not guarantee government support, particularly when large capital expenditures are constantly required.

According to figures gathered by the committee, funding for astronomy peaked in 1968 at about $227 million, and by 1972 (the latest year for which figures were available) it had shrunk to $187 million. And there are scant prospects of significant growth in the next few years, particularly in space astronomy. Recently, for example, the space shuttle has been taking up a growing share of NASA's stagnant budget and there is every reason to expect the trend to continue.

In view of the prospects of expanding graduate enrolment in astronomy and shrinking financial support for the science, the committee has suggested a number of controversial remedies.

● Most important, the rate of production of astronomers should be reduced, and "it is the responsibility of every university department which produces PhDs with specialisations in astronomy and astrophysics to assist in achieving this reduction", the committee states. All PhD students should be informed of the employment situation before embarking on graduate work, they should be carefully screened during the early years of their studies and the weaker ones should be weeded out. Goldberg also suggested that a limit should be placed on the number of universities offering PhDs in astronomy and astrophysics, so that as new departments are set up, older and less productive ones are phased out.

● To make astronomers more employable in other fields, the committee recommends that PhD astronomy

BRITAIN's nuclear power industry has been further streamlined with the announcement last week of the merger of Britain's two nuclear power groupings, British Nuclear Design and Construction (BNDC) and the Nuclear Power Group (TNPG), into one company, the Nuclear Power Company (NPC), which has been acquired by the National Nuclear Corporation (NNC). The government has agreed to make an *ex gratia* payment of up to £1.416 million to the shareholders of the now defunct companies for "unrecovered expenditure". Announcing the payment, Secretary of State for Energy Mr Varley said that the acquisition of the two consortia with their staffs by the NNC was "an important step forward". The acquisition includes the govern-

ment's 26% stake in British Nuclear Design.

The shareholding structure of the NPC and the names of the chief executives will be announced in the next few weeks, and it is expected that GEC, who were in favour of building the light water reactors rejected by the government last year, will drop its shareholding to 30% from the present 50%, with the UK Atomic Energy Authority increasing its stake from 15% to 30%.

The two existing bases, at Risley, Cheshire, where the design for the steam generating heavy water reactor is being carried out and Whetstone, Leicestershire, where work on the last two advanced gas-cooled reactors is continuing, are being retained for the "foreseeable future".

REPORTS from Moscow indicate that a new campaign is in preparation against the illicit "Sunday seminars" of *refusnik* scientists. Since the departure for Israel of Professor Aleksandr Voronel, founder of the seminar, the group has continued to meet, the venue alternating between the apartments of three leading members: mathematician Aleksandr Lunts, and physicists Viktor Brailovskii and Mark Azbel.

Of these three, Lunts has been threatened by the KGB with a show trial under Article 64 of the Soviet Penal Code, which relates to treason, which could result in a death sentence. The charge, phrased in deliberately vague terms, being that he had "undertaken a task on behalf of someone outside the Soviet Union for monetary gain".

Brailovskii, while not directly threatened, was called in for a discussion with Investigator Aleksandrov, who informed him of the charges being prepared against two other dissidents, dentist Mark Nashpits and engineer Boris Tsitlionik.

Mark Azbel, so far, does not seem to have attracted the attention of the authorities to the same degree, although there has been the usual routine harassment, and being a theoretical physicist, he is making a determined effort to continue his academic work, although cut off from all libraries and other facilities. The events of the last few years, however—arrests, hunger-strike and the nervous stress caused by constant uncertainty—has resulted in considerable deterioration of his health (he is suffering from severe cholelithiasis with reflex attacks of angina pectoris), and any increased pressure from the authorities could have very serious consequences.

● A DECREE of the Central Committee of the Communist Party of the Soviet Union (CPSU), published on March 21, 1975, states that the Jubilee Meeting of the Academy of Sciences of the USSR will be held in October 1975.

This meeting, originally scheduled for May 1974, was postponed, ostensibly on the grounds that further preparatory work was needed, although, at the time, the decision to cancel the planned international gathering was widely assumed to be connected with the concern expressed by Western scientists about the lack of academic freedom in the USSR and, in particular, the plight of Soviet Jewish scientists wishing to emigrate to Israel. This assumption was reinforced by the notice of postponement, which suggested that the celebrations would now be aimed at the grass roots of Soviet society.

The text of the new decree, after commenting on the successful local celebrations held throughout the Soviet Union on the theme "The achievements of science are for the national economy", states that the Jubilee Session of the Academy will be held "in Moscow, in October this year, with the participation of a Party, Soviet and community organisations, representatives of workers, scientists from the Academies of Science of the [constituent] republics, the Academy of Medical Sciences of the USSR, the Lenin All-Union Academy of Agricultural Sciences and the Academy of Pedagogic Sciences of the USSR. The Academy of Sciences of the USSR is given the task, together with the Jubilee Committee, of working out and presenting to the Central Committee of the CPSU proposals connected with the accomplishment of the celebrations of the Jubilee of the Academy of Sciences of the USSR.

"The Central Committee of the CPSU expresses its assurance that the Jubilee Session of the Academy of Sciences of the USSR in honour of the 250th anniversary of its foundation will be interpreted as a nation-wide festival, and will be conducted under the badge of a review of the achievements of Soviet science and the mobilisation of scientific teams for the solution of the problems proposed by the Twenty-fourth Party Congress on the acceleration of the rates of scientific and technological progress, ensuring the economic might of our homeland, strengthening the defensive potential of the country, raising the material prosperity of the workers, consolidating peace, and strengthening friendship b e t w e e n nations."

courses should be broadened to include experience in teaching, computer applications and electronics. "It is up to the entire community of astronomers to correct the image that astronomers are able only to look through a telescope and ponder the riddles of the universe," the committee adds.

● To help create more jobs for astronomers, the committee recommends that undergraduate astronomy programmes should be expanded. At present, the bulk of the astronomy community is employed in departments which award PhDs, and very few are to be found in undergraduate departments and colleges; the expansion of undergraduate astronomy courses would therefore help open up teaching posts. Coupled with that suggestion, the committee recommends that astronomy centres should establish arrangements for carrying out collaborative projects with astronomers in more 'isolated' research environments—such as undergraduate departments and liberal arts colleges.

Although there is considerable financial incentive for universities to carry on expanding their postgraduate departments, Goldberg said he has "considerable optimism that the recommendations will be taken up" and he pointed out that Harvard has already cut enrolment in astronomy PhD courses. □

Sweden's nuclear power game

from Wendy Barnaby

SWEDEN's nuclear future has been decided—officially until 1985. The ruling Social Democrats' energy plan, put to Parliament last week and expected to be approved, provides for the construction of nine reactors. With the four already in operation, Sweden will have thirteen reactors with a total electrical generating capacity of about 9,500 MW by 1985. This will be only two more than previously planned, but the increase is seen by critics as the thin end whose wedge will be an existing government plan to build 24 reactors by 1990. Construction on that scale would make the Swedes the world's greatest per capita users of nuclear power.

The plan is a piece of politics worthy of Prime Minister Olof Palme's considerable skills. Forced last year by public concern over the nuclear programme to postpone a decision on those plants not then under construction, he has with the present proposal given the appearance of responsible caution while allowing his government a reconsideration in 1978 of the ways energy is to be provided for 1985–90. By 1978, he explained, there would be more knowledge available on which to make a better-informed decision. By 1978, he could have added, the next parliamentary election will be safely out of the way with the emotional energy issue securely pegged for future consideration.

On the face of it, nuclear power seems an obvious choice for Sweden. Although the country has no cheap uranium resources (that is, uranium which can be mined for less than $10 a pound), it has almost half the non-communist world's known supplies of uranium in the next price range—$10–15 a pound. It has been estimated that the supplies of cheap uranium may become scarce in the mid-1980s, partly because of the eight-year time lapse between discovery and production of deposits. Unless exploration is stepped up, therefore, the more expensive uranium could come into demand at about the same time as the Swedes' suspended nuclear programme would have been—and perhaps will still be—well advanced. Sweden would expect to be a major exporter of reactor fuel elements. But the nuclear issue has caused an extraordinarily vigorous public debate over the past year, focused mainly on the safety aspects and the disposal of nuclear waste. (In fact Sweden neatly solves its radioactive waste problems by sending its reactor fuel elements to Windscale, England, for reprocessing.) More recently the social and security consequences of a nuclear

decision have come into focus. Pronuclear groups have insisted that no rise in the standard of living will be possible without large scale nuclear power, and have pointed out the dangers of dependence on foreign energy sources. Anti-nuclear forces have responded that the choice is not between increased growth and alternative energy sources: both are possible.

The two new reactors proposed by the government are officially justified on the grounds that they, together with increased hydroelectric power, will make up the extra 15 billion kWh of electricity necessary for a projected increase of 2% a year in energy consumption. Non-oil-fired power stations are being emphasised in an attempt to lessen the country's dependence on the Middle East. As the average annual increase in consumption over the 15 years until 1973 was 4.5%, the new projection will certainly test the efficacy of save-energy campaigns. It is hoped that, by 1990, growth in energy consumption will be zero.

As well as changing the relative emphasis on present energy sources, the plan provides money for research into fusion, geothermal, wind and solar power. But the fact that the allocations for this research are roughly only 10% of those for the building of new reactors shows where the government's confidence lies. It will be surprising if, having laid the groundwork so skilfully, the Social Democrats do not use the 1978 review to hasten the day when the Swedes will be the largest users of nuclear energy in the world. □

OECD energy

THE Organisation for Economic Co-operation and Development (OECD) report on the problems and perspectives of energy research and development published earlier this year provides a comprehensive and relatively up to date (September 1974) review of expenditure on all facets of energy research throughout the OECD countries, which include Europe, the United States, Canada, Australia, New Zealand and Japan.

Although the report does not commit itself to specific criticisms of member states' energy research programmes, it obviously feels the need for a longer term approach to the problem, which appears to be lacking in many aspects of energy research programmes set up in response to the 'energy crisis' of 1973. It warns for instance that the sudden upsurge in energy R&D must not be subjected to cutbacks once the most spectacular effects of the crisis have receded and the problem has become less sensitive politically.

In general the distribution of resources should be directed to keeping

as many alternative sources of energy open as possible. Research aimed at energy production should no longer be limited to one primary source. Perhaps the country which has made the largest turnaround is the United States which now supports a massive and diverse programme on every conceivable aspect of energy production but which previously had based its energy policy very largely on the availability of cheap imported oil.

One important and as yet relatively undeveloped field of energy research is energy systems. This covers the inter-relationships between production, transport and use of energy and also takes in any factors which might have a bearing on the smooth functioning of that system, such as effects on the environment and supply of skilled manpower. The use of energy accounting to clarify the energy flow through these systems from the level of primary energy up to the finished product is also encouraged. The study of energy systems, says OECD, can be an extremely important factor in moulding future policies.

Although the tables of statistics and research programmes are necessarily incomplete and sometimes countries cannot be directly compared as some figures include a measure of industrial research whereas some pertain to government expenditure only, they provide interesting reading. The USA of course leads the field spending over $1,000 million in the fiscal year 1974. France and Germany spent around $350 million and $450 million respectively and the United Kingdom spent around $228 million in 1973–74.

With regard to the organisation of energy policy, the OECD picks out Britain and the United States as the only two countries which have set up a new ministry or agency to deal with the complete problem, thus fixing a policy course that links energy R&D administration with general energy policies. In other countries, energy research has been taken under the wing of the science ministries, where they exist, or handed to some *ad hoc* committee.

Where energy problems can be reduced to matters of technology to be solved by a specific research programme, the outlook seems fairly optimistic. The member states of OECD, comprising as they do the most highly developed industrialised societies, are orientated to cope relatively easily with that type of problem. But energy is an extremely delicate political subject and affects the whole nature of society. Therefore, says OECD, "future development [of energy] will mainly depend on the political decisions of countries with regard to the nature of their economic growth and social structures". □

THE decision made last year that Britain's next commercial reactors should be of the steam generating heavy water type (SGHWRs) has given a predictable fillip to morale at the United Kingdom Atomic Energy Authority's research establishment at Winfrith, Dorset, where the 100-MW prototype has been supplying electricity to the grid for more than seven years. And not suprisingly, a greater proportion of Winfrith's annual spending of £9 million (of which £5 million represents payment for the electricity Winfrith supplies to the national grid) is being channelled into work on the SGHWR, at the expense of some of the establishment's other activities.

The Winfrith SGHWR has the rare distinction of being based on a design for a reactor more than five times its size, which means that scaling up to a commercial reactor of perhaps 660 MW (a size which matches the capacity of the electricity generating equipment now widely used in Britain) should be child's play by comparison with the same exercise for the British advanced gas cooled reactors (AGRs); the AGR programme in Britain is hopelessly behind time and still far from complete.

The secret is that an SGHWR is really a collection of mini-reactors of about 1 MW strung together. As the diagram shows, the basic building block, a pressure tube assembly, is remarkably simple: water at high pressure enters at the bottom and emerges from the top as steam which is fed to the turbines. Put 104 of them together in parallel and you have the Winfrith reactor, or a reactor of almost any desired size by varying the number.
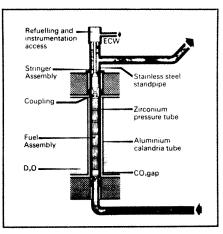
Although Winfrith will continue to be interested in the kind of research that is best carried out on a prototype reactor—testing of new component designs and materials, for example—it will be for the Nuclear Power Company to finalise the design details for the first commercial SGHWR.

Presumably at that time, too, people will start to talk again about the prospects for exporting SGHWRs. Although the potential customers of a few years ago, such as Australia, Greece and Finland, are probably lost, it is at least now possible to say as part of a sales pitch that electricity utilities in Britain are also buying them.

● MORE than a year since energy policy became a hot parliamentary property, the British Secretary of State for Energy, Eric Varley, continues the tradition of the committees under his purview by appearing before Commons sub-committees, armed with good intentions. When he first appeared before the Energy Resources Sub-committee of the Select Committee on Science and Technology, Mr Varley said last year that since he had only been at the job for three months it was early days for him to be formulating hard and fast policy. But he promised he would do his swotting up while listening to all kinds of advice. A little later his Advisory Council on Energy Conservation came before the same sub-committee and said that they too were open to suggestions, though they had precious

Energy in Britain

SGHWR boiling channel

few of their own to offer. Then followed, earlier this year, Varley's new Chief Scientist, Harwell *wunderkind* Walter Marshall, who virtually burst at the seams with enthusiasm while regretting that it was yet, alas, too soon to be expecting any energy blueprint.

Now Mr Varley has made a return appearance before the Commons energy group, who left him with what one Member of Parliament described as a flea in his ear. Which is to say they gave him to understand that they were not entirely bowled over by the progress of the Labour government's energy strategy. Reviewing the state of the game, Mr Varley harped on his December campaign of exhortation once too often for Labour member Mr Ronald Brown. The 'Save It' campaign was 40-year-old thinking, Mr Brown suggested. And the Energy Secretary's plea that people should economise by keeping their refrigerators three-quarters full was "pedestrian".

Mr Brown went so far as to suggest that the energy chief was in thrall to bodies of important chaps who were so busy beavering away at their uncoordinated committees that the whole energy package was turning out to be a "hotch potch". The chosen big-wigs were generally firm about the things which could not be done, but less firm about suggesting what could be done, said Brown, naming Ieuan Maddock (Chief Scientist at the Department of Industry) as the "godfather" of the gang. Mr Brown asked whether or not Mr Varley had the right people on the job (the energy conservation group are in fact part-timers) and the committee chairman, Arthur Palmer, confessed he had the distinct impression that Chief Scientist Marshall had too much on his plate, what with running Harwell and sitting on all those committees.

Not so, replied the Secretary of State, stoutly. Marshall wasn't complaining about overwork, and the advisory boards were manned by chaps with enormous experience. If they didn't appear to be pulling up trees it was worth bearing in mind that only 1% of energy used in the country was under government control, the rest being used by private industries which the government could simply advise. Alas, Mr Varley was leading with his chin. Apparently, one of the Department's advisory groups, the Advisory Council for Research and Development of Fuel and Power (ACORD) has been busying itself with the energy problems of the nationalised industries only, when its brief placed no such restriction on its activities.

When this was pointed out to Mr Varley, along with a suggestion that it was unrealistic to plan national energy policy by investigating only the nationalised concerns, the Secretary of State looked moderately sick and conceded that it was "a very good point" which had not been brought home to him before, and which he would look into.

As for the statistics, which caused him to brighten, at the outset, Mr Varley reported oil imports running recently at a yearly rate of less than 100 million tons, with the likelihood of even lower levels to come. This figure compared with 112 million tons in 1973 and 109 millions tons in 1974. Total energy consumption in 1974 was 4.5% down on the previous year and inland deliveries of oil in January this year were 4% down on the figure for 1974 and nearly 13% less than for January 1973. Petrol consumption, which normally rises at an annual rate of 7% was 5% down last January on the figure for January two years earlier.

But the committee wanted to know how much of this cut-back was positively caused by implementation of the Department of Energy's fuel-saving policy, and how much was caused by the general industrial recession. Not possible to say, claimed Mr Varley, but he promised to have somebody make the attempt. Certainly it can't all have been caused by a sudden overstocking of refrigerators.

correspondence

More on nutrition

SIR,—Since I participated in what Rivers says may have been the "golden age" of nutrition, I feel that I should comment on his indictment (January 10). He says that since 1945, "nutrition in the UK has been neither of military importance nor Nobel Prize ranking." The first circumstance is difficult to deplore since it mainly results from the lack of World War III; the second criticism is tinged with nostalgia. Rivers apparently expects nutritionists to produce miraculous "cures" of "obesity, diabetes mellitus, kwashiorkior, ischaemic heart disease and many of the other great nutritional [sic] scourges." He suggests that the reason that these cures have not appeared is the lack of an experimental animal with an evolutionary defect such as the loss of L-gulonolactone oxidase in the guinea pig. In other words, he thinks that these "scourges" are deficiency diseases awaiting the discovery of an ascorbic acid. But much obesity results from over-eating, only occasionally aggravated by endocrine imbalance; diabetes mellitus comes from hormonal insufficiency; and kwashiorkor from economic, cultural and environmental deprivation. Whether ischaemic heart disease is a nutritional deficiency is not known.

Rivers says that "we do not know why nutrition education fails when advertising works". But, alas, we do and this sentence is the keynote to Rivers' lack of understanding of the predicament of nutrition. This is the day of the nutritional pied piper. The story of scientific nutrition is too humdrum to attract popular attention, at least in the USA, in the fierce light of competition from the writings and speeches of Adelle Davis, Carlton Fredericks, Linus Pauling, Jerome Rodale, Doctor Atkins, R. J. Williams and the brothers Shute who promise relief from heart disease, cancer, infections, prostate trouble, obesity, shortness of life, and back pains by devouring pumpkin seeds, raw milk, dried seaweed, ascorbic acid, fertilised eggs, apricot seeds, vitamin E and garlic, or by not consuming white bread, pasteurised milk and food additives. As Philip White of the American Medical Association says, "what usually happens is that the scientific spokesman ends up sounding like a close-minded Establishment bore who wants to suppress bright new ideas".

Nutritionists wearily spend their time trying to pick up the pieces in the wake of this ideological hurricane. Yes, we know too well that advertising works. Rivers kindly informs us that we "are not in business to pursue uncluttered academic research" (where have scientists heard this before and how does Rivers expect a remedy for ischaemic heart disease without research?) "but to improve the nutritional status of the population", to do which we "don't know how". It sounds as if he doesn't know what he is talking about. One thing we do not need is more Nobel laureates, especially loquacious ones. We have the tremendous task of applying the knowledge of nutrition to the needs of a species that is fantastically eccentric and variable in its attitude towards the acceptability of food, and that is increasingly influenced by nutritional nonsense dispensed by the mass media. The task is made frustrating by the fact that the annual bulk cost for supplying the needed allowances for ten vitamins and eight essential minerals is less than $1 per capita, but for socio-economic political and logistic reasons, we cannot get them to most people. On top of this, we are faced by the awesome world food crisis. There is little helpfulness in Rivers's fuzzy sermonising. There is need for calories and there is no alternative to photosynthesis.

THOMAS H. JUKES
Berkeley, California

Peaceful explosions

SIR, — That the Non-Proliferation Treaty has defects, to some of which you have drawn attention, is widely accepted, but to adapt a well-known phrase, it is the best treaty we have. Roughly two-thirds of UN states are full parties to the treaty, which requires states to refrain from policies many of them would otherwise actively pursue.

Your particular complaint that nothing has been done to implement the requirements of Article V, that there should be an international organisation for the provision of peaceful nuclear explosions to non-nuclear weapon states, is not strictly correct. On the recommendation of the Conference of Non-Nuclear Weapon States in 1968, the UN General Assembly agreed in 1971 that the International Atomic Energy Agency itself should assume that responsibility. So far Czechoslovakia, Madagascar and Romania have enquired about services,

but a specific proposal is yet to come.

This does not mean that peaceful nuclear explosions will not be a contentious issue at the Review Conference in May, but the issues will be different from those you suggest. Now that Congress has for practical purposes put an end to the Plowshare programme, and as it becomes clearer that the Russian programme of peaceful explosions, however extensive, is yielding disappointing results, the nuclear powers will be tempted to suggest that non-nuclear parties should forego peaceful explosions. Politically that would be dangerous; let non-nuclear states discover economic reality for themselves.

That the treaty is for practical purposes innocent of security assurances for non-nuclear weapon states is true enough, as your leading article says, but it could hardly be otherwise. In practice, the best way of achieving this goal is along the lines of the Latin-American denuclearisation treaty (the Treaty of Tlatelolco, 1967). Indeed, there is good evidence that states unwilling to accede to the NPT will take part in regional arrangements like this. Although the main defect of the treaty is, as you say, that it asks for "minimal concessions" from the nuclear powers, it does require of them rapid progress towards a comprehensive test-ban treaty and measures for the reduction of nuclear stockpiles. Indeed, the NPT is a compact between non-nuclear states and nuclear states in which the former undertake to refrain from "horizontal" proliferation and the latter to pursue nuclear arms control. Much of the argument at the review conference will no doubt hinge on the extent to which the superpowers can fob off their critics with the Threshold Test-Ban agreement and SALT II. Probably they will not succeed. There will be pressure for more effective means of monitoring the super-powers than five-year reviews provide.

Precisely for this reason, the NPT is a potentially important forum within which measures of nuclear arms control can be developed. At the same time, there are many significant ways in which the inequalities of the treaty can be softened, not necessarily within its legal structure. To "suggest the treaty be scrapped and new approaches tried" is to run the risk of setting back arms control for a decade or more.

JOHN MADDOX
London EC4, UK

news and views

Excitement over β₂-microglobulin
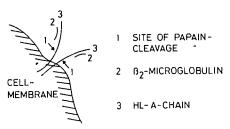
from Martin C Raff

FROM its meagre beginnings in 1968 when it was isolated from the urine of patients with renal tubule dysfunction (Berggard and Bearn, *J biol Chem*, **243**, 4095), β₂-microglobulin has rapidly blossomed into a molecule of great interest and importance to immunologists It is synthesised and secreted by most nucleated cells of vertebrates (Nilsson *et al*, *Nature new Biol*, **244**, 44, 1973) and is also present on the surface of these cells, as well as in the urine, serum (1–2 mg l⁻¹) and other body fluids Although its function is still unknown, recent observations have markedly sharpened interest in this ubiquitous protein by suggesting an evolutionary link between β₂-microglobulin, immunoglobulin (Ig) and the major histocompatibility (H) antigens

The complete amino acid sequence of human β₂-microglobulin has been determined and shows a striking degree of homology with the constant domains of human IgG (Cunningham *et al*, *Biochemistry*, **12**, 4811, 1973) It has 100 amino acid residues, a molecular weight of about 11,500, a single intrachain disulphide bond which forms an intrachain loop of similar size to those seen in the homology regions of Ig, and contains no carbohydrate These findings led to the suggestion that β₂-microglobulin represented a free Ig domain with effector function similar to the terminal domain (C_H3) of IgG, which it resembles most closely (Peterson *et al*, *Proc natn Acad Sci USA*, **69**, 1697, 1972)

Two independent lines of evidence have indicated that β₂-microglobulin is an integral part of the subunit structure of all the membrane proteins that carry the serologically defined major histocompatibility antigens (H-2 in mouse HL-A in man) on the surface of virtually all normal nucleated cells When HL-A (Cresswell *et al*, *Proc natn Acad Sci USA*, **70**, 1603, 1973, Tanigaki *et al*, *Immunology*, **26**, 155, 1974) and H-2 (Rask *et al*, *Nature*, **249**, 833, 1974) antigens are solubilised by detergent or proteolytic enzyme treatment of cells, they are found to be composed of a heavy chain (~34,000 or 44,000 daltons depending on whether papain or detergent is used respectively)

and a light chain (~12,000 daltons) which appears to be identical with β₂-microglobulin There is evidence that both HL-A (Strominger *et al*, *Transplantn Rev*, **21**, 126, 1974) and H-2 (Rask *et al*, *Transplantn Rev*, **21**, 85, 1974) are four-chain structures in the membrane, with two heavy chains covalently linked by disulphide bonding between their hydrophobic tails, and the two β₂-microglobulin molecules non-covalently bound to the heavy

1	SITE OF PAPAIN-CLEAVAGE
2	β₂-MICROGLOBULIN
3	HL-A-CHAIN

Rask's tentative picture of an HL-A molecule in the cell membrane (from *Transplantn Rev*, **21**, 102, 1974)

chains, a structure remarkably similar to Ig Characterisation of solubilised H antigens in guinea pig (Finkelman *et al*, *J exp Med*, **141**, 27, 1975), rat and rhesus monkey (Tanigaki and Pressman, *Transplantn Rev*, **21**, 15, 1974) have suggested that the β₂-microglobulin–H association is a basic feature of H antigenic molecules in mammals

A different approach to determining associations on the cell surface involves the binding of multivalent ligands (such as antibodies) to specific determinants (antigens or receptors) on the surface of intact cells to induce their redistribution into 'patches' or 'caps', and then studying the distribution of other membrane determinants In such co-capping experiments, most antigens and receptors that have been studied redistribute independently an exception being that all the HL-A antigens on human lymphocytes (Poulik *et al*, *Science*, **182**, 1352, 1973, Solheim and Thorsby, *Tissue Antigens* **4**, 83, 1974, Ostberg *et al*, *Nature*, **249**, 463, 1974, Neauport-Sautes *et al*, *J exp Med*, **139**, 957, 1974) and other cell types (Solheim, *Transplantn Rev*, **21**, 35, 1974) co-cap with β₂-microglobulin when the cells are treated with anti-

β₂-microglobulin antibodies These experiments make it clear that the association of solubilised HL-A with β₂-microglobulin in not an artifact of the solubilisation and/or separation procedures Capping HL-A with anti-HL-A antibodies fails to cap all of the β₂-microglobulin, suggesting that not all of the β₂-microglobulin is associated with HL-A

Although β₂-microglobulin occurs free in the serum, its structure makes it unlikely that it can be bound in the membrane other than by interacting with other membrane proteins In mice two other plasma membrane proteins recently have been found to be associated with β₂-microglobulin, the thymus leukaemia (TL) antigens (Ostberg *et al*, *Nature*, **253**, 735, 1975), which are restricted to thymus lymphocytes and lymphoma cells, and the teratocarcinoma alloantigen(s) (Artz and Vitetta, personal communication), which is restricted largely to early cleavage and morulae stages of mouse embryos, spermatozoa, and teratocarcinoma cells It is interesting that both of these alloantigens are determined by genes that are closely linked to the H-2 complex

On the other hand, not all alloantigens coded for by genes in the H-2 complex are associated with β₂-microglobulin, the Ia (immune response associated) alloantigens, determined by genes in the IR region of the H-2 complex, seem not to be associated with β₂-microglobulin In those cases where membrane polypeptides are normally associated with β₂-microglobulin, it is unlikely that β₂-microglobulin is required for their insertion or maintenance in the membrane This is suggested by the finding that a human lymphoblastoid cell line from a patient with Burkitts' lymphoma (Daudi) does not synthesise β₂-microglobulin or have it on its surface and yet probably carries the heavy HL-A polypeptide chain on its plasma membrane (Rask *et al*, *Transplantn Rev* **21**, 85, 1974) But it is possible that the β₂-microglobulin serves to stabilise the functional conformation of these polypeptides (whatever their function turns out to be) The structural linkage of β₂-micro-

globulin with a variety of membrane proteins controlled by genes in or near the major histocompatibility complex raises the question of whether the gene for β_2-microglobulin is also located in this chromosomal region The report by Goodfellow *et al* (*Nature,* **253**, 267, 1975) indicates that in man at least it is not By studying a variety of human–mouse somatic cell hybrids they found that cells carrying human chromosome 15 expressed human β_2-microglobulin on their surface, while cells not containing this chromosome did not, in a line which carried chromosome 15 as the only human chromosome, human β_2-microglobulin was expressed Since the HL-A complex has been assigned previously to chromosome 6, it seems virtually certain that in man, the β_2-microglobulin and HL-A genes are unlinked

Despite the apparent lack of genetic linkage, the physical association of β_2-microglobulin with H or H-linked, membrane-bound gene products is intriguing Taken together with the striking homology in structure between β_2-microglobulin and some Ig domains, it suggests the possibility that β_2-microglobulin, Ig and possibly these histocompatibility-linked genes have evolved from a common ancestral gene The association of unlinked but evolutionarily related gene products to form a membrane subunit protein has a well established precedent in membrane bound Ig, where unlinked light (L) and heavy (H) chain gene products make up the subunit molecule H_2L_2 Since membrane-bound Ig is known to function as an antigen-specific receptor on B lymphocytes, it may be that these other membrane molecules also have some kind of recognition or receptor role

In the case of Ig both L and H chains have separately coded constant (C) and variable (V) regions, with V_L and V_H both contributing to the antigen-combining site, thus the multichain structure serves the obvious purpose of greatly increasing the potential number of combining sites that V_L and V_H gene products can form Since there is no evidence for variability in the structure of β_2-microglobulin, the grand purpose of the H–β_2-microglobulin multichain structure remains a mystery Unfortunately, very little is known about the structure of the histocompatibility heavy chains, and the possibility of their having separate constant and variable regions, or regions homologous to Ig domains $\pm$ β_2-microglobulin, remains open

The gene products of the H complex are mainly cell surface proteins, many of which are involved in various immune responses, particularly T lymphocyte responses, such as graft rejection, graft-versus-host responses, mixed lym-

phocyte reactions, cell mediated lymphocytotoxicity and other specific immune responses to a variety of antigens Although the nature of the antigen-specific T cell receptor(s) remains controversial, and although it is not known how these H-complex gene products function, the physical association of β_2-microglobulin with some of these surface proteins provides a suggestive evolutionary link between B cell-mediated humoral immunity and T cell-determined cell-mediated immunity The possible immunological importance of some of the lymphocyte membrane proteins associated with β_2-microglobulin is underlined by the recent findings that anti-β_2-microglobulin antibodies inhibit various T cell responses (Solheim, *Transplantn Rev* **21**, 35, 1974) and are mitogenic for B cells (Maller and Persson, *Scand, J Immun,* **3**, 100, 1974)

It is not surprising that immunologists are excited about β_2-microglobulin

Element 106 and onwards?

from C J Batty

THE recent announcements by both Soviet and American teams of the discovery of element 106 is not only a further chapter in the saga of rival claims to be first but raises the interesting question as to how many other elements remain to be discovered, and in particular, what are the chances of producing superheavy elements

To some extent the rival claims are due to the differences in techniques used by the Soviet and American workers G N Flerov and his co-workers at the Joint Institute for Nuclear Research at Dubna near Moscow have generally used nuclear reactions involving fairly heavy ions and have looked for spontaneous fission activity On the other hand A Ghiorso and the team at the University of California, Berkeley, San Francisco have used heavier targets such as Californium, lighter incident ions and have made very detailed studies of the resulting α-activity In both cases the techniques are difficult and the resulting activities short lived, with typical half lives of a few seconds or less As a sign of the rival claims elements 104 and 105 have been christened Rutherfordium and Hahnium in the USA and Kurchatovium and Nielsbohrium in the USSR Perhaps in an atmosphere of detente, element 106 so far remains unnamed

Details of the Soviet work so far seem to be only available in unpublished reports but it involves the use of a radically different reaction method Rather than using the heaviest target material available they instead chose lead The nucleus ^{208}Pb has the advantage that it has 'magic' numbers

of both neutrons and protons and as a result the compound nucleus formed may have a low excitation energy This leads to a decrease in the number of neutrnos emitted and an increase in the cross section for forming the residual nucleus in its ground state The use of lead rather than fissile material also helps in reducing unwanted background activities The technique has been very successfully used by the Dubna group (*Nuclear Physics*, A239,. 157, 1975) in producing two new isotopes of element 104 using the ^{208}Pb $(^{50}$Ti,2n) ^{256}104 and ^{207}Pb$(^{50}$Ti,2n$)^{255}$104 reactions In the work on element 106 they used a beam of ^{54}Cr ions and the team believe they have seen the ^{208}Pb$(^{54}$Cr,3n$)^{259}$106 reaction with the final nucleus decaying by spontaneous fission with a half life of between 4 and $10\,\text{ms}^{-1}$

The work at Berkeley (*Phys Rev Lett*, **33**, 1490, 1974) used a ^{249}Cf target, as in the previous experiments on elements 104 and 105, together with a beam of ^{18}O ions from the 'SuperHILAC' accelerator to study the ^{249}Cf$(^{18}$O,4n$)^{263}$106 reaction Using a sophisticated system of α-detectors linked to an on-line computer they not only observed the α-decay of ^{263}106 with an energy of 9 06 MeV and half life of $0\,9 \pm 0\,2$ s but also observed the α decay of the daughter nucleus ^{259}104 $(t_{\frac{1}{2}} = 3$ s, $E\alpha = 8\,8$ MeV) and of the grand-daughter ^{255}No $(t_{\frac{1}{2}} = 3$ min, $E\alpha = 8\,1$ MeV) so establishing the decay sequence

$$^{263}106 \xrightarrow{\alpha} {}^{259}104 \xrightarrow{\alpha} {}^{255}\text{No} \xrightarrow{\alpha}$$

With very small cross sections for forming these very heavy nuclei together with their short half lives for decay it is clearly going to be difficult to go to the even heavier elements But it is understood that the Dubna team are now trying to produce other isotopes of element 106 using their new technique and may attempt to synthesise elements 108 and 110 using beams of ^{58}Fe and ^{64}Ni ions No doubt the Berkeley team have their own plans for work in this tough but exciting field

Which leaves us with the question of the superheavy elements These are an island of elements with atomic numbers in the region of 114 which owe their possible existence to the extra binding energy associated with the closed nucleon shells at 114 protons and 184 neutrons Prediction of their half lives for decay is difficult but some theories have suggested values sufficiently long to warrant searching for them in natural materials on Earth (*Nature phys Sci*, **246**, 26, 1973) so far generally without success

One problem with trying to produce these superheavy nuclei using the techniques employed for the 104 to 106 region is their very large neutron excess so that for example ^{298}114 cannot be obtained by the complete fusion of any real combination of target and projectile Nevertheless the Dubna group have reported (*Sov J nucl Phys* **19**, 123, 1974, *Sov J nucl*

Phys **19**, 247, 1974) experiments using beams of Zn and Ge ions, the latter being accelerated by a tandem arrangement of two cyclotrons Looking for spontaneous fission activity with an experimental arrangement which permitted high sensitivity, the teams were unable to find any evidence for the production of superheavies It seems that further work will require even more sensitive experiments and higher intensity beams of energetic heavy ions

Solar gravitational deflection of radio waves

from J M Riley

SINCE the publication of Einstein's general theory of relativity in 1916, there have been many attempts to test the prediction of this theory that electromagnetic radiation is deflected in the gravitational field of the Sun The experimental test of the prediction involves measuring the change in the apparent position of a star when it is close to the Sun The effect is extremely small as the predicted deflection amounts to a maximum of 1 75 arc s, for a ray path from a star which grazes the limb of the Sun, and decreases inversely with distance from the centre of the Sun A very accurate measurement of this deflection is required as it is clearly of fundamental importance to find out how closely general relativity agrees with observation and to test its predictions against those of other theories of gravitation Among the alternative theories which have received serious consideration is the Brans–Dicke scalar–tensor theory which, in its latest form, predicts that the deflection is 0 94 times that predicted by general relativity

Until ten years ago the only tests had been carried out optically during total solar eclipses and, owing to great technical difficulties, had provided only semi-quantitative support for general relativity with accuracies of about 25% Recently however it has become possible to perform the experiment at radio wavelengths using the technique of interferometry, in which the position of a radio source may be found by measuring the difference between the time of arrival of signals from the source at two radio antennae some distance apart on the Earth's surface, this method involves knowing with great precision the time at which the signals are recorded at each antenna An experiment of this type has been performed several times since 1969 using the QSO 3C279 which passes behind the Sun in October every year,

it may be observed for several days before and after this when it is very close to the Sun The change in its apparent position is measured relative to that of another source, 3C273, which is close to it in the sky but sufficiently far from the Sun during the experiment to be unaffected by the gravitational deflection

The first tests of this kind were made using antennae separated by relatively short distances (from 1 to 5 km) operating at frequencies between 2,700 and 8,000 MHz, it was therefore possible to have direct electrical connection between the antennae and consequently it was relatively easy to standardise the time of the observations at the two antennae The most accurate experiment of this type gave a result 0 96±0 05 times the value predicted by general relativity

It is also possible to carry out the experiment using antennae several hundreds of kilometres apart In such an experiment the difference between the time of arrival of signals at the two antennae is proportionately greater than in the shorter baseline experiments and in principle therefore the experiment should be much more accurate But in a long baseline experiment, direct electrical connection between the antennae is impossible so that independent clocks must be used at the two sites, due to instabilities in these clocks there is then a problem standardising the time of the observations at the two antennae Furthermore, as the two sites are so far apart there are likely to be very significant differences in the path lengths through the atmosphere of the radiation arriving at the two antennae

In a recent paper in *Physical Review Letters* (**33**, 1621, 1974) Counselman *et al* describe the first results of such an experiment performed in October 1972 using a baseline of 845 km and a frequency of 8,000 MHz In this experiment the problem of the differences between the independent standards used at the two sites was overcome by using a pair of antennae at each site, one directed at 3C279 and the other at 3C273 A single clock governed the recording of the signals received by both antennae at a given site, the difference between the time of arrival of the signals from 3C279 at the two sites could then be measured with reference to that between the time of arrival of the signals from 3C273 at the sites, thereby eliminating the effects of any clock instabilities at either place This method also reduces the effects of any differences in the atmosphere and ionosphere above the two sites In analysing the data it was necessary to assume models for the atmosphere, ionosphere and the solar corona, and uncertainties in these models, particu-

larly in that for the corona, contribute about half the error in the final result. The remaining error is attributed to experimental difficulties, in particular those introduced by fluctuations in the solar corona The observed deflection was 0 99±0 03 times the value predicted by general relativity This measurement is probably the most accurate yet made of the gravitational deflection of radiation and the result is in close agreement with general relativity, it is slightly more than one standard deviation from the value predicted by the Brans–Dicke Theory

The prospects for improving the accuracy of this test of general relativity using the method of very long baseline interferometry are good, as it is possible to eliminate several of the problems encountered in the experiment mentioned above by making simultaneous observations at two frequencies It is likely that accuracies better than 1% may be achieved in the very near future

A hundred years ago

WHITE'S "SELBORNE"

White's Natural History of Selborne Edited by J E Harting, F L S Illustrated by Bewick (London Bickers and Co, 1875)

ALTHOUGH we have no evidence that, within the last century, there has been any considerable change in the average standard of human mental power amongst civilised nations, the surroundings of every day life have so greatly altered, both in their quality and in the rapidity of their occurrence, that the standard of ordinary existence has undergone a corresponding modification The introduction of steam loco motion, the electric telegraph, and the penny post have developed such a condition of unrest in humanity at large that the unalloyed repose of a continuous rural life is rarely sought for, and as infrequently obtainable We can hardly conceive it possible that anyone, such as a life-fellow of a college, as was Gilbert White, of Oriel, Oxford, should at the present day settle down in any out-of-the-way part of the country, satisfied with nothing more than an opportunity of observing and recording the surrounding phenomena of nature More would be expected of him, and he would be continually led to feel that he was but one of the instances of the vegetating influence of an antiquated system, whose advantages were being daily disproved by his individual existence

from *Nature*, **11**, 423, April 1, 1875

Causes of climatic change
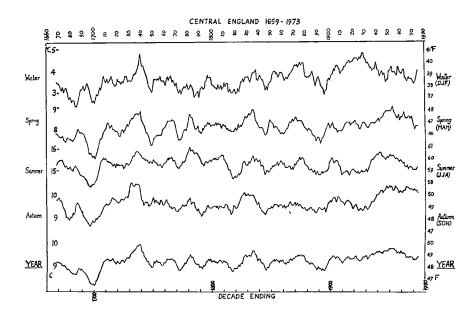
M K Miles of the Meteorological Office reviews the recent evidence while (below) John Gribbin reports on a lecture which may have helped to draw fresh ideas to the field

Decadal running averages of seasonal and annual mean temperatures, from Manley, *Q J R Met Soc*, **100**, 402, 1974

FROM 1880, when the first reasonably reliable determination of the average temperature of the Northern Hemisphere was possible, there has been a warming (with only minor reverses) until 1940 and since then a cooling In both cases the effect has been most marked north of about 70°N latitude and in the winter half of the year From the 300-year-long temperature series prepared by Manley (*Q J R Met Soc*, **100**, 389–405, 1974) for central England it could be said that winter temperatures have been rising since the end of the seventeenth century, at a rate of 0 36 °C per century with reverses lasting several decades This temperature series indeed shows fluctuations on all time scales but there seem to be no clearly marked periodicities (see figure)

Possible effects of sunspots

Many investigators have sought a relationship with the various periodicities displayed by sunspot activity, and although for some records and some seasons statistically significant results have been obtained, there are also contradictory results If there is a real relation with the 11-year sunspot cycle it is a complicated one, changing its phase relationship from epoch to epoch, but in any case not accounting for a very substantial amount of the variance at that time scale The same applies to the double sunspot cycle (successive 11-year cycles showing opposite magnetic polarity of the sunspots)

A relation with a longer cycle of solar spot activity varying between 60 and 100 years has been claimed, but the amplitude of the response is small and not clearly above the play of chance There is naturally even less convincing evidence for a suggested periodicity of 180–200 years related to a solar cycle whose length and physical characteristics are not well established The ¹⁸O record from the Camp Century ice core has been adduced as evidence for periods of 77 and 177 years, but adjustments were made to the time scale to preserve persistent climatic oscillations so it is not entirely independent evidence Even so the record is substantially out of step with the

Manley temperature record for central England in the eighteenth and nineteenth centuries The variations of ¹⁴C content in dated wood specimens have been claimed to show 200- and 400-year periodicities, but the experimental error in the ¹⁴C determinations means that such a claim should be accepted with great reserve

It is still not certain that the heat output from the Sun varies enough with changes in the number of sunspots to affect the lower troposphere Satellite measurements indicate large changes in the ultraviolet part of the spectrum at different times during the 11-year sunspot cycle, but the fraction of the total energy in this part of the spectrum is very low To date satellites have not monitored changes in the spectral range 0 3 to 1 0 μm

Terrestrial influences

Other factors thought capable of producing changes in the lower troposphere are dust (volcanic and wind erosion dust), CO_2, water vapour and changes in ocean temperature and salinity Increasing CO_2 from burning of fossil fuel is generally estimated to be capable of explaining a substantial part of the warming since 1880, and a clearer stratosphere resulting from fewer volcanic eruptions in the twentieth century compared with the eighteenth and nineteenth would be expected to act in the same sense If this be so then a strong cooling factor must have come into play since 1940 The first major eruptions after Katmai in 1912 did not occur until the early 1950s, so a sudden increase in dust from other sources (and aerosol loading) has been postulated Measurements of the electrical conductivity of the air over

the Atlantic indicate an increase in the number of Aitken nuclei in recent years compared with the early 1930s but the data are not adequate to show whether this happened around 1940 or not On the other hand direct solar radiation measurements as collated by Budyko and Vinnikov (*Glav, Uprav Gidromet, Sluz, Met Gidr*, **9**, 3–13, 1973) do not reveal a substantial drop until about 1945 The dust from semi-arid zones in America and the Soviet Union which one would expect to have increased following the dry conditions of the 1930s does not seem to have affected radiation

Aerosols from increasing industrial activity and changing agricultural practices are probably the most likely agents to have contributed to the fall in direct solar radiation said to have occurred since 1945 There are, however, two factors to be considered before it may be concluded that the cooling is due to this First, the aerosol being largely in the troposphere will often lie below cloud top, and does not have a long residence time in the atmosphere Second, it may absorb radiation as well as reflecting it so that the net cooling effect may be very small However, it must be said that attempts to determine the secular change of atmospheric turbidity do not all support an increasing global trend either for the time since the late 1950s or for the century

Quite small variations in the global cloud cover are capable of altering the heat balance substantially but data are not adequate to reveal any long term trend Similarly data about ocean temperature and salinity are not adequate to assess the part the oceans might have played in the warming and subsequent cooling Minor changes in

oceanic circulation are capable of leading to substantial changes in the heat exchange with the atmosphere

The area of ice and snow cover in the polar regions decreased between 1900 and 1930, then began to increase in the late 1950s One school of thought attributes the increase to the lower direct heat input since about 1945, but the decline in the Atlantic winter circulation in the last two to three decades could perhaps have produced the same effect There is even some evidence that a further change occurred about 1970 The area of sea ice was less for the four years 1971–74 than for the preceding four years Again there has been a change in the Atlantic winter circulation but it is noteworthy that Budyko in an updating in 1973 of his direct radiation index shows an increase setting in during the late 1960s

Other atmospheric variation

Changes in precipitation have also received attention—local changes as well as regional distribution—and various claims have been made for periodic fluctuations The supporting evidence has not however been strong enough to lead to a consensus of

opinion For example the 22 years of the double sunspot cycle is claimed (and widely accepted) to be related to the incidence of droughts over the plains of the American Middle West but data for the steppe and forested zones of the Soviet Union do not show the same pattern

Fluctuations in the strength of the general wind circulation in the Northern Hemisphere broadly parallel the temperature behaviour though there is an indication of a lead of about 10 years—the peak in the hemispheric westerlies for the latitude band 35–55°N being reached by 1930 The North Atlantic winter circulation which displays these changes to a marked degree certainly declined after 1930 whatever latitude band is examined It is difficult to relate these or the smaller scale variations in a sensible way with changes in the temperature gradient between the tropics and the pole or suspected changes of the meridional heat flux Only a small fraction of the internal energy of the atmosphere is converted to kinetic energy and so there is no reason to expect a simple relation between energy input and circulation energy

The reality of strictly periodic fluc-

tuations in the Earth's climate being to some extent in doubt and trends in various parameters being either doubtful (as for example turbidity) or not yet distinguishable from random fluctuations, satisfactory predictions for the next few decades cannot be made Perhaps all that is justified are estimates of the range of possible future fluctuations based on past climatic data together with assessments of the influence of human activity on the surface of the Earth and the composition of its atmosphere

Protecting damaged plant life of Galapagos

from Peter D Moore

THE impact of man and his domesticated animals upon the unique flora and fauna of the Galapagos Islands has been the source of considerable concern in recent years Schofield (*Biol Cons*, **5**, 48, 1973) considered that many endemic trees and shrubs, such as *Scalesia pedunculata* and *Miconia robinsoniana*, were threatened both as a

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On March 10, Dr Peter Wright (University of East Anglia) spoke at the University of Bristol about climatic change to an audience of mixed physical scientists He emphasised that climate is always changing, on many timescales, and that these changes occur globally A disturbance of climate in one part of the world will affect the whole system, with possibly drastic repercussions on the other side of the globe But man's direct influence on climate, through heating of cities, for example, seems insufficient to disturb the global machinery The kind of disturbance cited by Wright as important would be about 1,000 km across and persistent—paving over the interior of Brazil, say, or smaller and more energetic, but persistent—exploding a nuclear device in the same spot every day for ten years So it is still the study of 'natural' climatic fluctuations which is of key importance

Wright dismissed most of the proposed natural causes of change as minor contributors Solar variations (over the sunspot or longer cycles) could play a part, but are unlikely to dominate to the extent of causing ice ages, the Milankovitch theory that changes in the Earth's orbit and inclination affect insolation enough to cause ice ages seems more tenable (especially now that the geological record has been re-

New climatologists begin here

interpreted to give a better guide to the variations of glaciation over the past few million years), dust in the atmosphere from volcanoes or blown away from eroded and desert areas is a good candidate for irregular effects, but is difficult to reconcile with cyclic changes in climate (if, that is, you believe that climatic changes are cyclic, which Wright does not), geomagnetic fluctuations could, says Wright, 'just conceivably' play a small part (others might argue that by varying the flux of cosmic rays reaching the atmosphere such changes in magnetism could be of greater importance), and man's influences have certainly not been important as yet

All of these processes are forcing effects, Wright, however, believes that, although such variations may make conditions more suitable for, say, icier conditions, it is feedback that maintains any climatic regime

For example if a patch of ocean in the northern hemisphere becomes warmer than average, the effect will tend to be to shift the circum-hemispherical wind streams so that instead of blowing from west to east across the warm patch they swing

up from the south-west, pass around the north of the warm patch and swing away to the south-east The effect is to bring warmer air over the 'anomaly', tending to maintain its warmer state

A more realistic example of a feedback system is the "Southern Oscillation", in which a patch of ocean along the equator just west of the Americas fluctuates between a colder and less cold state (the difference is no more than 3 °C) with associated changes in the pattern of trade winds Each state seems stable, and each is maintained by feedback between wind and ocean systems Why should the situation ever change? Clearly there are external factors involved, even in the rest of the air–ocean system, and computer modelling is far from the stage of sophistication where all these effects are included

As Wright pointed out, climatology today is still to a great extent in the data gathering stage, but already many data are available for analysis and more physical ideas are needed for testing both in the real world and in the models—and this is where there seems to be scope for new talent Outsiders with experience in fluid mechanics, thermodynamics, statistical interpretation of noisy data and so on could play a vital part in the interpretation of the data as they are collected

result of intensive clearance and cultivation of upland areas and because of the spread of exotic plant species introduced by man (see *Nature*, **242**, 299, 1973) Davies (*Nature*, **249**, 788, 1974) pointed out the threat to the islands' flora presented by feral goats and the problems in carrying out scientific research and conservation on the inadequate budget of the Charles Darwin Research Station Can one be sure that the demise of the Galapagos flora and fauna will even be recorded adequately?

A research programme to monitor habitat changes in the islands was instigated by the research station in 1966 Permanent plots of between 6 25 m² and 100 m² were established on several islands in habitats which were thought to be threatened, and recordings have been made since that time of vegetational changes within the plots Hamann (*Biol Cons*, **7**, 37, 1975) has now presented some of the data emerging from this study over the period 1966–73

The arid zone vegetation has suffered to different degrees on the various islands On Pinta, where goats have in the past been a most serious pest, the denudation of vegetation has been severe, sometimes resulting in soil erosion Recent reduction in goat population density has not yet resulted in any marked recovery of the vegetation There is however cause for optimism in view of the regeneration which has been noted on Santa Fé, where feral goats were exterminated in 1971 *Cordia lutea, Encelia hispida, Lantana peduncularis* and *Scalesia helleri* are all recovering in the arid zone of this island since the cessation of grazing One might hope that regeneration will occur in Pinta also, if goat populations can be further reduced

The *Scalesia pedunculata* forests of Isla Santa Cruz, although badly affected by grazing and clearance, seem to have the capacity for rapid recovery and growth under suitable conditions Recent intensive hunting of introduced mammals by National Park wardens may be responsible for the observed increase in saplings of *Scalesia* within the plots However, cattle seem to be very partial to young *Scalesia* trees, so full recovery can only occur if cattle grazing is controlled Exclosure plots could supply the answer to this

Perhaps the most sensitive vegetation type of the Galapagos is that dominated by *Miconia robinsoniana* with *Cyathea weatherbyana* Much of this zone has been damaged by burning, followed by cattle grazing, and this has resulted in its replacement by ferns, particularly *Pteridium aquilinum* It may be that recent prolonged droughts have also contributed to the failure of regeneration in *Miconia* This vegetation type will undoubtedly provide the most difficult conservation problem of all on the Galapagos It has almost disappeared from San Cristóbal and is failing to recover on Santa Cruz, its only other station Protection of the zone from further burning and grazing must be regarded as a management priority

The control of introduced mammals on the Galapagos has proved possible and worthwhile It is to be hoped that funds will be found to continue and intensify this programme of conservation and that schemes can be initiated which will satisfy the needs of both farmer and conservationist on the islands

Long period of lunar crater formation

from Peter J Smith

ONE of the hopes not entirely fulfilled by the Apollo missions was that it would be possible to recover a significant number of lunar rocks older than 4,000 million years As terrestrial rocks of corresponding age have never been found, some workers had hoped that access to lunar material would enable important light to be thrown on the first 500–600 million years' evolution not only of the Moon itself but also of the Solar System particularly the Earth Unfortunately, most lunar mare and highland basalts dated radiometrically appear to be between 3,950 and 3,130 million years old It is true that some highland rocks, notably the cataclastic anorthosites, have yielded ages much closer to that of the Solar System, but some doubt has been expressed as to what such great rock ages really mean The difficulty is that much of the lunar regolith in the appropriate regions represents ejecta from the major basins, material which has been severely shocked, and it is not entirely clear just how shock affects a rock's radioactive properties

Recently, however, Gooley *et al* (*Geochim Cosmochim Acta*, **38**, 1329, 1974) described an unshocked troctolite (Apollo 17) comprising plagioclase, olivine and bronzite, which they conclude cooled at a rate of a few tens of degrees per million years at a depth of 10–30 km Two whole rock samples and a plagioclase separate from this material have now been dated by Husain and Schaeffer (*Geophys Res Lett*, **2**, 29, 1975) using the ^{40}Ar-^{39}Ar technique Extensive tests and close agreement between the ages obtained from all three samples convince Husain and Schaeffer that the accurate mean age of the troctolite is 4,260±20 million years

The corresponding ^{38}Ar/Ca cosmic ray exposure age is 156±8 Myr, suggesting that the troctolite was last brought to the surface comparatively recently The much older ^{40}Ar-^{39}Ar age, on the other hand, supports the major conclusion from Gooley and his colleagues that the rock was originally a product of large scale igneous processes which formed the early lunar crust The combined evidence from petrography and the Ar systematics is that the troctolite probably formed about 4,500 million years ago and then cooled very slowly at depth for tens to hundreds of millions of years During most of this phase the temperature was apparently sufficiently high to make the rock an open Ar system But for the past 4,260 Myr the rock has evidently been a closed Ar system, thus enabling one to date the time at which it was excavated from depth and reburied under an ejecta blanket

This is the simplest and most reasonable chronology consistent with the available data But Husain and Schaeffer believe that they can go even further in relating the rock's history to named events on the Moon For (shocked) lunar breccias from Apollos 14–17, the histogram of measured ^{40}Ar-^{39}Ar ages (see, for example, Schaeffer and Husain, *Geochim Cosmochim Acta*, Suppl **4**, 1847, 1973) covers the range 3,900–4,260 million years and is sharply peaked at 3,900–4,000 Myr As the breccias were almost certainly formed by impacts on the lunar surface and as extensive Imbrium ejecta exist at the Apollo 14–17 landing sites, the histogram peak strongly supports the view that the Imbrium event occurred between 3,900 and 4,000 million years ago

If this is so, the older breccias (assuming their measured ages to be reliable) and the Apollo 17 trocolite must be related to earlier events As far as the trocolite is concerned, the impact which excavated it is now identified by Husain and Schaeffer as the Serenitatis event, largely because the Apollo 17 site lies at the rim of the Serenitatis basin If this is correct, it follows that the age of the Serenitatis is probably 4,260 Myr, the age of the troctolite This, in turn, suggests that what Husain and Schaeffer term the "lunar basin forming era" lasted at least 300 Myr (Serenitatis–Imbrium) In fact it may have lasted much longer because the Serenitatis impact event was preceeded by the Fecunditatis, Tranquillitatis and Nubium events In any case it now appears that these major lunar basins could not all have been formed in a relatively short time about 4,000 million years ago, as some workers have suggested

review article

Schlieren experiment 300 years ago

J. Rienitz*

Schlieren techniques for making visible density gradients in transparent media seem to be older than had been supposed. Robert Hooke developed a method of this kind as early as 1672 but, unlike Christian Huygens, who described a similar technique in 1685, obviously aroused no interest.

DURING the long decades of his activity as Curator of Experiments of the Royal Society of London, Dr Robert Hooke (1635–1703) developed a large number of ideas, experiments, and instruments in the fields of science, technology, and medicine[1,2] Lack of time to elaborate many of his ideas, undeserved discredit, the change in the conception of scholarship during the Newtonian Age, and rivalries in the edition of his bequeathed papers have meant that much of his work has remained unknown One of his unrecognised achievements seems to have been the development in 1672 of a true schlieren method Commonly, it is Léon Foucault (1859) and August Toepler (1864) who are regarded as the discoverers of this group of techniques[3,4]

After the invention of the microscope and the telescope at the beginning of the seventeenth century activity in the field of optics became widespread Refraction was investigated thoroughly, methods for improving the lenses as well as the performance of the optical instruments in general were sought, and the reflections of scholars on the nature of light began to gain impetus Hooke's interest in astronomical problems, as well as in the mechanics of gases, led him to investigate atmospheric refraction He treated it extensively in Observation LVIII of his famous *Micrographia* of 1665 together with a number of related phenomena, writing, for example, "This I have likewise often observ'd in a hot Sunshiny Summer's day, that looking on an Object over a hot stone, or dry hot earth, I have found the Object to be undulated or shaken, "[5] His explanation is quite correct "a multiplicate refraction, caused by the unequal *density* of the constituent parts of the *medium,* whereby the motion, action or progress of the Ray of light is hindred from proceeding in a streight line, and *inflected* or *deflected* by a curve"[5]

These density gradients in a transparent medium are termed streaks Hooke mentions streaks in gases and vapours, in fluids, and in solids The unequal refraction which is the consequence of the unequal optical density is barely visible to the naked eye, and hence the development of schlieren techniques which enhance the contrast Robert Hooke mentioned his schlieren set-up first when he was treating the combustion of a candle as a part of his extensive but often overlooked[1,6] investigations on the nature of combustion, in the course of which, incidentally, he clearly realised the function of oxygen[1] The experiment was performed by Hooke before the Royal Society on February 22, 1671 old style (or March 4, 1672, Gregorian calendar) "to shew, that, besides the flame and smoke of a

candle there is a continual stream rising up from it, distinct from the air"[7] A week later, the experiment "was repeated and proved satisfactory"[7] Finally, on March 14, Hooke submitted an account of the experiment which was registered[7] Here, he describes first the method (For sake of clarity the notations of the illustration are inserted in the following original text in square brackets)

"I took a large concave reflecting glass, or a large convex refracting glass [L], and so placed in respect to my eye [E], that a candle [C₁] set at a certain distance beyond the refracting glass, or between the eye and the superficies of the reflecting glass, enlightened the whole area of the said glasses in respect to the eye Then continuing to keep the eye in that place, where the area of the said glasses appeared to be wholly filled with the flame of the candle, I caused another candle [C₂] to be placed very near the said glasses, between the eye and the glass, or beyond also, if I made use of the refracting glass [L] Then looking stedfastly at the flame of this last candle, it was very plain to be perceived, that the flame thereof was encompassed with a stream of liquor, which seemed to issue out of the wick, and to ascend up in a continual current, or *jet d'eau,* to keep itself intire, and unmixt with the antient air, notwithstanding that it was a considerable way carried above the aforesaid flame"[7] Then he mentions "several turnings, whirlings, or vortices in the ambient air" familiar to all who ever have observed a burning candle in a schlieren apparatus In Fig 1, S may designate a region of streaks in homogeneous surroundings Owing to the gradient of refraction the bundle of rays R penetrating this inhomogeneity will be deflected more or less from the pupil of the eye E so that the streaks stand out more or less dark against the bright background The pupil of the eye plays here, in principle, the same role as the knife edge in the methods of Foucault and Toepler[4,8],
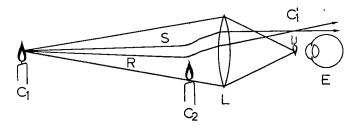
Fig 1 A reconstruction of Robert Hooke's schlieren set-up C₁ the first (illuminating) candle, L the lens, E the eye of the observer, C'₁ the image of C₁ produced by L, in reality at the pupil of E, C₂ the second candle, S a region of streaks above C₂, R a bundle of rays of light passing through S For clarity the distances of C₁ and E from L are represented as smaller than they may actually have been

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but at present schlieren systems with a circular cutoff instead of the knife edge are also used[3]

Hooke explained the operation of his method in almost the same way "The manifestation of this phoenomena [*sic*] was from differing refractions of the body of the jet d'eau from that of the ambient air for the flame of the first candle being but small, and placed at a considerable distance from the refracting, or reflecting glass, the smallest variation in the refraction of the medium between the first glass and the eye caused the darkness to intermix with the light, so to exhibit the appearance of the heterogeneous jet d'eau"[7]

These words show, too, that Hooke obviously realised at least one of the conditions mentioned by Mach[8] for ensuring the sensitivity of the schlieren method that is, a small light source The subsequent passage which states that the first candle is "placed at a considerable distance from the refracting, or reflecting glass" is, however, ambiguous It could simply refer to long focal distances, another condition of sensitivity mentioned by Mach[8] On the other hand, it could refer to the first condition and signify that the flame of the first candle is imaged on the pupil of the eye at a reduced scale (Fig 1) Since even a small candle flame has an area greater than that of the pupil, the area of its image may by these means be made comparable with that of the pupil, so that a minute difference of refraction at a certain region of the object causes at least partial non-coincidence of both areas and hence a darkening in the image of that region This seems, however, to be valid only for the lens, for in the case of the mirror this interpretation contradicts the passage cited above which says that the first candle should be placed "between the eye and the superficies of the reflecting glass"

After having submitted his account, Hooke "promised to exhibit at the next meeting, an experiment to shew a phaenomenon not unlike this, to be produced by several bodies dissolved in oil of vitriol"[7] On March 28 of the same year he performed an experiment of dissolving salnitre in common water Here, a stream is observed "composed of water and of the particles of nitre dissolved therein" which "was here descending, as in the former experiment a stream or fluid produced by a candle dissolved by the air ascended"[7] This seems to be the experiment promised by Hooke, but he may have been mistaken or changed his mind about the appropriate substances Later on he demonstrated experiments on mingling oil of vitriol and water, but here no observations of streaks are reported

Hooke mentioned his schlieren experiment once more in his Cutlerian Lecture entitled "Lampas", which was published in 1677, although so briefly that it is recognisable only by those familiar with it "This Figure and shape of the flame and vapours may be plainly seen by the help of a Metalline Concave placed at a certain distance and Position, and also by observing the shadow of the candle cast by the beams of the Sun upon a sheet of white Paper, or white Wall, but the way of a Concave *speculum* is incomparably beyond it, because it doth so very plainly shew the form and manner of the streams rising above "[9] The second, less sensitive procedure mentioned here is the same in principle as the shadowgraph method introduced into schlieren optics by Dvorak in 1880[3,10] The same effect can be observed when the sun is shining through blown window panes

Finally, Hooke repeated his experiment before the Royal Society on January 16, 1678 (or January 26, 1679, Gregorian calendar), and the short report ends "This was plainly seen by the president and divers others of the members present to their satisfaction"[7] At the same date Hooke records briefly in his diary "Shewn experiment of flame"[8]

At much the same time another schlieren experiment had been performed by Christian Huygens[11] He illuminated the back of a convex lens with the reflected light of a candle in a way similar to Hooke's method, observing the reflection in order to test the surface for defects of polish, as well as the glass for streaks These defects of polish are local irregularities in the surface which, analogous to streaks, deflect the light into another direction than their surroundings and can therefore be made visible in the same way by schlieren methods With lenses of great focal length Huygens even used a small telescope to observe the schlieren image, a fact Toepler emphasises as an advantage of his method compared with that of Foucault[4]

Huygens certainly developed his method without knowledge of Hooke's procedure, for if he had been acquainted with it, he would not have hesitated to repeat the experiment with the candle which is still today one of the most spectacular demonstrations in schlieren optics He seems to start from his thinking of the laws of image formation in terms of the "point de confusion maximum" (point of maximal blurring), by which he means simply the image point of an object If one observes a candle through a lens, the image of the candle becomes increasingly blurred as the eye moves back from the lens At the point mentioned above the blurring reaches a maximum because the whole aperture of the lens is filled with light and the image of the candle is lost As the distance of the eye from the lens increases, the image becomes clearer once more and finally, one is able to discern an inverted image of the candle Hooke's description of his method shows that he was familiar with this observation too

This concept has been used by Huygens in his *La Dioptrique*[12] published in 1653, but his schlieren method was evidently invented much later It was first published posthumously in 1703, but the editors of his *Oeuvres Complètes* fix it at 1685[11], 13 years after Hooke This date seems to be justified, for in a letter to his brother Constantyn who had often made telescope lenses with him, dated October 7, 1686, Christian Huygens described his method as if it were new and added the words "Je voudrois que vous fussiez icy pour estre present a cette epreuve"[13] (I wished you were here in order to be present at that test)

Huygens' method, though rarely mentioned in literature, has obviously not been completely forgotten by the experts, as has been the fate of Hooke's findings The well known glassmaker Pierre Louis Guinand, who collaborated later on with Fraunhofer, reported that he became acquainted with it in 1798 when he was visiting the opticians at Paris[14] It seems very probable that the news of Huygens' method reached Paris through the well known work *A compleat System of Opticks* by Robert Smith[15], first published in 1738 Huygens's treatise had originally been written in Dutch, but was later translated into Latin (by Hermann Boerhave, according to Abraham Gotthelf Kastner in the German translation of Smith's book) Smith certainly made Huygens's ideas better accessible by reporting them in English Since, however, the very skilled optician Mitouflet Thomin in Paris was obviously ignorant of these techniques in 1749[16], one may assume that it was the publication of the French translation of Smith's work in 1767[17] that changed the situation A connection between the knowledgeability of the opticians at Paris and Léon Foucault may thus not be completely excluded

The relatively detailed accounts of Hooke concerning streaks, as well as his schlieren set-up, are essential for interpreting the scanty and rare hints at microscopical methods related to schlieren techniques that have been dropped by Hooke and some other old microscopists Such investigations are now in progress

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articles

Possible power source of Seyfert galaxies and QSOs

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The possible presence of massive black holes in the nuclei of galaxies has been suggested many times In addition, there is considerable observational evidence for high stellar densities in these nuclei I show that the tidal breakup of stars passing within the Roche limit of a black hole initiates a chain of events that may explain many of the observed principal characteristics of QSOs and the nuclei of Seyfert galaxies

STARS of mean density ρ are broken apart by the tidal force of a black hole of mass M if they pass within a distance

$$R_T \simeq (6M/\pi\rho)^{1/3} \tag{1}$$

of it They are physically absorbed by the black hole if they pass it within the gravitational radius,

$$R_G = 2GM/c^2 = 2\,95 \text{ km } (M/M_\odot) \tag{2}$$

R_T and R_T/R_G as functions of M are given in Table 1 I have assumed solar type stars in evaluating ρ in equation (1) For $M < 3 \times 10^8 M_\odot$, the tidal radius is greater than the gravitational radius so that stars are far more likely to be broken apart by the black hole than absorbed whole By the time the black hole has grown to the critical mass $M_c \simeq 3 \times 10^8 M_\odot$, it may have produced several times its own mass in gas, by breaking up stars This may be the source of the net outflow of gas observed in the nuclei of many galaxies

Capture of gas by the black hole

Much of the gas produced by tidal breakup is captured and subsequently accreted by the black hole The tidal breakup of a star requires energy which is ultimately supplied by a reduction in the kinetic energy of the star with respect to the black hole The energy required to break up a star of mass m and radius R is

$$E_b = \tfrac{3}{4}(Gm^2/R) \tag{3}$$

for a polytrope of index $n = 3$ The gas produced by the breakup of a solar type star is captured into a bound orbit by the black hole if the velocity of the star with respect to the black hole

before their encounter was less than 535 km s^{-1} Thus if the velocity dispersion of the stars in the nucleus of a galaxy is less than about 500 km s^{-1}, much of the gas produced by tidal breakup is captured and ultimately accreted by the black hole

For the larger black holes, which have $R_T \sim R_G$, emission of gravitational radiation may also be important in reducing the kinetic energy of the star with respect to the black hole[1], but I shall not consider this additional loss mechanism here

The gas produced by the breakup of a particular star may travel in a bound orbit to a large distance from the black hole, but eventually it must return to the original periastron distance of its stellar precursor The mean semi-major of the gas produced by the breakup of a solar type star by a black hole of mass M is

$$r = (2M/3m)R[1 - 2\langle V^2 \rangle R/3Gm]^{-1}$$
$$= 1\,5 \text{ pc } (M/10^8 M_\odot)[1 - \langle V^2 \rangle/(535 \text{ km s}^{-1})^2]^{-1} \tag{4}$$

where $\langle V^2 \rangle$ is the mean-squared velocity of the stars in the galactic nucleus before their encounter with the black hole Thus a $10^6 M_\odot$ black hole is surrounded by a massive gas cloud with a typical radius of 10^{-2} pc The dust in this cloud strongly absorbs the ultraviolet radiation emitted by the gas being accreted by the blackhole This may be responsible for the infrared emission of QSOs and Seyferts The ionised gas in this large cloud may in turn be responsible for the forbidden-line radiation from QSOs

Collisions among the gas clouds originating from the breakup of individual stars will release enormous amounts of energy High velocity collisions between gas clouds can explain several phenomena associated with QSOs (ref 2) The dissipation of energy from collisions reduces the effective semi-major axis of the gas until it is well within the Roche limit Ultimately the gas must be accreted by the black hole unless it is stopped by rotation Even if this occurs, the gyration radius of the gas must be much less than the Roche limit of the black hole unless the gas acquires additional angular momentum after the breakup of its stellar progenitors by interacting with the stars around the black hole If the angular momentum is sufficiently large that the gas settles into a disk spinning around the black hole, viscous forces eventually will allow most of this gas to be accreted by the black hole as in an X-ray binary[3,4] The accretion of gas by a massive black hole produces a large amount of ultraviolet radiation[4] which will tend to ionise the massive gas cloud around the black hole to produce the observed forbidden-line radiation and the cores of the permitted lines The Doppler broadening

Table 1 Breakup and accretion of stars by a black hole

M/M_O	R_T/R_O	R_T/R_G	L_E/L_O (Eddington Limit)	t_E (yr)	L/L_E ($\rho_s = 10^6 M_O$ pc^{-3})	t_D (yr)
10^1	4 31	$1\ 02\times10^5$	$3\ 2\times10^5$	$1\ 6\times10^9$	$1\ 5\times10^{-3}$	$1\ 8\times10^{11}$
10^2	9 28	$2\ 19\times10^4$	$3\ 2\times10^6$	$1\ 4\times10^9$	$3\ 2\times10^{-3}$	$8\ 4\times10^{10}$
10^3	20 0	$4\ 72\times10^3$	$3\ 2\times10^7$	$1\ 2\times10^9$	$7\ 0\times10^{-3}$	$3\ 9\times10^{10}$
10^4	43 1	$1\ 02\times10^3$	$3\ 2\times10^8$	$9\ 4\times10^8$	$1\ 5\times10^{-2}$	$1\ 8\times10^{10}$
10^5	92 8	$2\ 19\times10^2$	$3\ 2\times10^9$	$7\ 3\times10^8$	$3\ 2\times10^{-2}$	$7\ 9\times10^9$
10^6	200 0	$4\ 72\times10^1$	$3\ 2\times10^{10}$	$5\ 2\times10^8$	$7\ 0\times10^{-2}$	$3\ 3\times10^9$
10^7	431 0	$1\ 02\times10^1$	$3\ 2\times10^{11}$	$3\ 2\times10^8$	$1\ 5\times10^{-1}$	$1\ 2\times10^9$
10^8	928 0	2 19	$3\ 2\times10^{12}$	$1\ 1\times10^8$	$3\ 2\times10^{-1}$	$2\ 7\times10^8$
$3\ 2\times10^8$	1,374 0	1 00	$1\ 0\times10^{13}$	0	$4\ 8\times10^{-1}$	0

resulting from the rotation of the gaseous disk around the black hole and the large random velocities of dense colliding gas clouds near the black hole may be responsible for the large widths of the permitted emission lines in Seyferts and QSOs

Luminosity and total energy

It is evident that most of the mass acquired by a black hole less massive than $M = 3\times10^8 M_O$ is from the accretion of gas rather than of whole stars In this case the total energy released by this accretion is $E = 0\ 057\text{-}0\ 43 Mc^2$ depending on the angular momentum of the black hole[5] If $E = 0\ 2Mc^2$, the total energy released by the black hole by the time its mass reaches the critical value $M_c = 3\times10^8 M_O$ is $E_c = 1\ 1\times10^{62}$ erg $\equiv 9\times10^{20} L_O$ yr The maximum luminosity produced in the accretion of material by the black hole is given by the Eddington[6] limit,

$$L_E = 3\ 2\times10^4 L_O(M/M_O) \tag{5}$$

Thus the maximum possible luminosity produced by the accretion of gas by the black hole is $L = 10^{13} L_O$ which occurs when $M = M_c$ This is very near the peak observed luminosity of QSOs The total energy release could power a quasar at this luminosity for as long as 9×10^7 yr

If $E = 0\ 2Mc^2$, the rate of mass accretion by a black hole growing at the Eddington limit is

$$dM/dt_E = 1\ 1\times10^{-8}\ M_O\ \text{yr}^{-1}\ (M/M_O) \tag{6}$$

In this case, the time required for a black hole to grow from a mass M to $M_c = 3\times10^8 M_O$ is

$$t_E = 9\ 3\times10^7\ \ln(M_c/M)\,\text{yr} \tag{7}$$

Table 1 shows t_E and L_E as a function of M I assume that the luminosity of a QSO drops by orders of magnitude when its mass reaches $M = M_c$ at which point the stars are swallowed whole by the black hole rather than being broken apart tidally first

I note (Table 1) that a seed black hole growing at the Eddington rate takes about $1\ 6\times10^9$ yr to increase its mass from about $10 M_O$, a mass one may expect from stellar evolution, to $M_c = 3\times10^8 M_O$ So the observed number of QSOs in the Universe would decrease very rapidly after the Universe was more than $1\ 6\times10^9$ yr old If the Universe is presently $1\ 2\times10^{10}$ yr old, this critical time corresponds to $Z = 2\ 8$ in an Einstein–de Sitter universe This is consistent with the observations of Schmidt[7] that the density of QSOs in the Universe increases as $(1+Z)^6$ up to $Z \simeq 2\ 5$ and then it either remains constant or decreases for larger Z

Growth governed by stellar density

If the density of stars in the nucleus of a galaxy is not sufficiently large, this density rather than the Eddington limit governs the rate of growth and luminosity of the black hole Assuming

Newtonian mechanics, the rate at which gas is being produced by the breakup of stars passing within the Roche radius R_T of a black hole of mass M is

$$\frac{dM}{dt} = \rho_s\langle\sigma V\rangle$$

$$= 8\ 6\times10^{-16}\frac{M_O}{\text{yr}}\left[\frac{\rho_s}{M_O/\text{pc}^3}\right]\left[\frac{R_T}{R_O}\right]\left[\frac{M}{M_O}\right]\left[\frac{\text{km s}^{-1}}{\langle V^2\rangle^{1/2}}\right]$$

$$= 1\ 9\times10^{-16}\frac{M_O}{\text{yr}}\left[\frac{\rho_s}{M_O/\text{pc}^3}\right]\left[\frac{\text{g cm}^{-3}}{\rho}\right]^{1/3}\left[\frac{M}{M_O}\right]^{4/3}\times$$

$$\times\left[\frac{\text{km s}^{-1}}{\langle V^2\rangle^{1/2}}\right] \tag{8}$$

Here ρ_s is the stellar mass density in the vicinity of the black hole It is assumed that at very large distances from the black hole the stars have a Maxwellian distribution of velocities with an r m s velocity $\langle V^2\rangle^{1/2}$ In this derivation I have assumed that the mass and radius of a typical star broken apart by the black hole are much smaller than the mass and tidal radius of the black hole I have also made use of the fact that the escape velocity at a distance R_T from the black hole is very much greater than $\langle V^2\rangle^{1/2}$

If all the gas produced in the breakup of the stars is accreted by the black hole integrating equation (8) suggests that the time required for a black hole to grow from a mass M to a mass M_c is

$$t_D = 1\ 7\times10^{15}\,\text{yr}\left[\frac{M_O\,\text{pc}^{-3}}{\rho_s}\right]\left[\frac{M_O}{M}\right]^{1/3}\left[1-\left(\frac{M}{M_c}\right)^{1/3}\right]\times$$

$$\times\left[\frac{\langle V^2\rangle^{1/2}}{\text{km s}^{-1}}\right] \tag{9}$$

Here I have assumed solar type stars so that $\rho = 1\ 41$ g cm^{-3} Table 1 shows t_D as a function of M for a galactic nucleus in which $\rho_s = 10^6 M_O$ pc^{-3} and $\langle V^2\rangle^{1/2} = 225$ km s^{-1}, the observed velocity dispersion in the nucleus of M31 (ref 7) The table also gives the luminosity, L, in units of the Eddington luminosity, L_E Here I have used $L = 0\ 2\,(dM/dt)\,c^2$ I note that at a fixed M, $(t_D)^{-1} \propto L \propto \rho_s/\langle V^2\rangle^{1/2}$

Thus simple scaling gives L and t_D for a galactic nucleus with any other value of ρ_s and $\langle V^2\rangle^{1/2}$ It is not likely that $\langle V^2\rangle^{1/2}$ differs very much from one nucleus to another, but there are sizable differences in ρ_s Assuming $M/L = 10 M_O/L_O$, the data of Kinman[8] indicate that $\rho_s \simeq 10^5 M_O$ pc^{-3} within 1 pc of the nucleus of M31 For the nucleus of our Galaxy infrared observations[9] indicate that $\rho_s \simeq 7\times10^6 M_O$ pc^{-3} within 0 5 pc of its centre Schwarzschild[10] finds indirect evidence of $\rho_s = 2\ 2\times10^8 M_O$ pc^{-1} within 3 5 pc of the nucleus of

the Seyfert galaxy NGC4151 For a black hole to have had enough time to grow to $M_c = 3 \times 10^8 M_\odot$ within the age of the Universe (which I assume to be $1\,2 \times 10^{10}$ yr), the initial mass of the black hole had to exceed $M_i \simeq 10^7 M_\odot$ in the nucleus of M31, $M_i \sim 10^2 M_\odot$ in the nucleus of our Galaxy, and $M_i \lesssim 1 M_\odot$ in the nucleus of NGC4151 The very low value of M_i for NGC4151 suggests that Schwarzschild's estimate for ρ_s may be somewhat large From the values of L/L_E in Table 1, a black hole in the nucleus of M31 could never break up stars fast enough to allow it to radiate at the Eddington limit In our Galaxy a black hole would radiate at the Eddington limit when its mass is equal or greater than $M_E = 10^7 M_\odot$, for NGC4151 this occurs when $M_E = 10^2 M_\odot$ When $M > M_E$ the breakup of stars by the black hole produces gas faster than the Eddington limit allows it to be accreted by the black hole In these cases, we may expect the surplus gas to be driven out of the nucleus by radiation pressure Such outpouring of gas is observed in several Seyferts This mass loss can be prodigious If $\rho_s = 10^7 M_\odot$ pc^{-3}, the mass loss rate approaches $12\,5$ $M_\odot$ yr^{-1} as M approaches M_c The mass loss rate as well as the luminosity would then drop effectively to zero for $M > M_c$

If present ideas on the evolution of massive upper main sequence stars are correct, we may reasonably expect black holes with masses of about $M_i = 10 M_\odot$ to form in almost every galactic nucleus Such black holes would have had sufficient time in our Universe to grow to $M_c = 3 \times 10^8 M_\odot$ if the stellar density in the galactic nucleus exceeds $(\rho_s)_0 = 1\,5 \times 10^7 M_\odot$ pc^{-3} (Table 1) This watershed density is very sharply defined If ρ_s is even 2–3 times less dense than this, the minimum mass of a seed black hole which has sufficient time to grow to M_c in the age of the Universe increases sharply to 10^2–$10^3 M_\odot$ which is much greater than the masses of black holes formed by normal stellar evolution If $\rho_s > 2$–3 $(\rho_s)_0$, almost any black hole has time to grow to M_c Thus any QSO or Seyfert nuclei present today are likely to have a stellar density of about $1\,5 \times 10^7 M_\odot$ pc^{-3} or slightly greater At this density the black hole radiates at the Eddington limit for $M > M_E = 10^5 M_\odot$ If $\rho_s \geq 10^8$–$10^9 M_\odot$ pc^{-3} any black hole with $M > 10 M_\odot$ would grow at the rate governed by the Eddington limit These black holes would have reached $M = M_c$ at about $Z = 2\,8$ in the observable Universe Thus there should be a maximum in QSO activity at this epoch followed by a rapid decline for lower values of Z This is consistent with observations[7]

Two refinements may increase somewhat the rate at which stars are swallowed by the black hole One complication is the possible formation of a high density stellar cusp around a black hole embedded in a dense stellar nucleus[11] Another is the shrinking of the entire stellar nucleus as its stars are captured by the black hole How this arises can be seen by considering the effect of these captures on a typical star in the nucleus Some of the stars captured by the black hole are in orbits in which, on the average, they are further from the black hole than the test star At some point in time each of these stars crosses inside the orbit of the test star never to return Thus, as these stars are accreted by the black hole, the test star and the other remaining stars in the nucleus feel a progressively increasing gravitational potential To satisfy the virial theorem, the velocity dispersion of the stars in the nucleus must increase This added kinetic energy is supplied by a net shrinking of the effective radius, R_N, of the galactic nucleus The increase in the velocity dispersion of the stars in the nucleus would by itself decrease the rate of accretion by the black hole, but this is more than compensated by the increased stellar density in the nucleus I note that $\langle V^2 \rangle \propto R_N^{-1}$ while $\rho_s \propto R_N^{-3}$ (actually, $\rho_s \simeq R_N^{-2}$ when stars accreted by the black hole are considered) From equation (8), this results in a net increase in the rate of accretion by the black hole compared to the case where we ignore both the increased density and the increased velocity dispersion in the galactic nucleus This effect can only become important as the mass of the black hole approaches that of the galactic nucleus or $M \sim 10^8 M_\odot$

Orbit diffusion and relaxation time

In the previous section, particularly in the derivation of equation (8), I made the implicit assumption that the rate of orbit diffusion resulting from the random gravitational encounters between stars is sufficiently fast that at any given time the number of stars with orbits that pass within the Roche radius, R_T of the black hole is nearly the same as it would be if there was no breakup of these stars This is true as long as the total mass M_n of the stars in the galactic nucleus is large compared to the mass M of the black hole In the absence of orbit diffusion, the velocity distribution of the stars at a given point in the nucleus would be isotropic (if the stellar distribution is relaxed) except for those velocity directions corresponding to orbits which cross R_T On average, the fraction, F_v, of the velocity directions occupied by these orbits is the fraction of all stars in the nucleus that have orbits which intersect R_T Thus as long as $F_v \lesssim 1$, a very small amount of velocity randomisation is sufficient to fill in these small holes in the otherwise isotropic velocity distribution and consequently to repopulate the orbits that cross R_T In other words the time necessary to repopulate these orbits is very small compared to the relaxation time[12],

$$t_R = V^3/4\pi G^2 m^2 n \ln N \qquad (10)$$

which is the time necessary to completely rerandomise the velocities In the typical galactic nucleus of interest, $V = 225$ km s^{-1}, $n = 10^7$ stars pc^{-3}, $m = 1 M_\odot$, $M_n = 10^8 M_\odot$, and $N = M_n/m = 10^8$ In this case $t_R = 3 \times 10^8$ yr

The rate of orbit diffusion is sufficiently large to result in there being no significant depletion of the stars with orbits that cross R_T as long as the characteristic time for the consumption of all the stars in the galactic nucleus by the black hole is greater than the relaxation time, that is, the diffusion rate is sufficient as long as $M_n/|dM_n/dt| > t_r$ If $M_n \sim 10^8 M_\odot$, this condition is satisfied until the mass of the black hole has grown to $M \sim M_c$ Thus the assumption of an adequate rate of orbit diffusion implicit in the previous section is valid

A massive black hole at the galactic centre?

Any such object would have to be very modest to produce no more than the observed nonstellar radiation emitted from the nucleus If gas accretion by this object produces all the ionising radiation required to maintain the H II region at the galactic centre, the luminosity of this radiation is $L_i = 1\,9 \times 10^8 L_\odot$ (ref 13) if the energy per photon is 2 rydberg From Table 1, with $\rho_s = 7 \times 10^6$ $M_\odot$ pc^{-3}, this requires a black hole mass $M \simeq 4 \times 10^4 M_\odot$ Equation (3) requires that the radius of the cloud of gas and dust produced by the breakup of stars by a black hole of this mass be $\langle r \rangle \simeq 10^{-3}$ pc This is at least consistent with the observed "point" infrared source at the galactic centre which has a radius of less than $0\,1$ pc and a luminosity of 3×10^5 $L_\odot$ (ref 8) $M \simeq 4 \times 10^4$ $M_\odot$ is likely to be a firm upper limit on M The ionisation could be due to something else If M is this large, it would only take another $1\,5 \times 10^9$ yr to grow to M_c It is unlikely (but of course not impossible) that we would catch the black hole just at this crucial stage in its growth A more troublesome point is the fact that the initial mass of this object had to be about $10^2 M_\odot$ to have reached $M = 4 \times 10^4$ by the present epoch This initial mass seems rather large from the standpoint of stellar evolution If the luminosity of the infrared source results from the accretion of stars broken up by the central black hole, its mass must be at least $M \simeq 3 \times 10^2 M_\odot$ This is probably the minimum mass of the central black hole The initial mass of this black hole need only have been slightly greater than $10 M_\odot$ to have allowed it to grow to $3 \times 10^2 M_\odot$ in $1\,2 \times 10^{10}$ yr It will take another 10^{10} yr for such a black hole to grow to $M = M_c$

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Dredged basalts from the mid-oceanic ridge north of Iceland

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An examination of the concentrations of the incompatible elements in tholeiites from the Kolbeinsey Ridge indicates that plume magmas have probably not overflowed into that area from Iceland or Jan Mayen Island The results also show that the chemistry of plume magmas may be intimately related to regional geochemical patterns, and it is possible that Iceland represents a 'weak spot', or decompression focus, which causes complex flow patterns within and from the asthenosphere

WE present here petrological data on dredge samples recovered from the mid-oceanic Kolbeinsey Ridge north of Iceland Most of the materials described were recovered during two cruises in 1972–73 in the North Atlantic

Iceland and Jan Mayen Island represent subaerial expressions of recent volcanism associated with the Iceland Plateau and Kolbeinsey Ridge Iceland consists of an extensive, largely tholeiitic, flood-basalt plateau built up in about the last 30 Myr and cut by three neovolcanic zones containing tholeiitic, alkalic and other basalt types[1-3] Its great volumes of lava, their high discharge rates and relatively high contents of K, Ti, P, rare earth elements (REE), $^{87}Sr/^{86}Sr$ and so on have been considered expressions of a hot, rising mantle plume[2,4-6]

The older Faeroes and East Greenland basalts also have K, P, and Ti values comparable to those found in Iceland[7], and this may be[8] the result of plume activity in the early Tertiary

Jan Mayen Island consists of ankaramites, alkali basalts and their differentiates together with their explosive equivalents, tholeiitic flows are unknown Subaerial material only dates back to 0 5 Myr BP but construction of the island's base could be assigned to the mid-Tertiary or early Pleistocene[9,10] A plume origin has also been proposed for Jan Mayen Island[11] The two islands lie at the limits of the Iceland Plateau, a generally smooth (presumably basaltic) platform at a depth of less than 1 km (refs 12 and 13) Transecting this plateau is the Kolbeinsey Ridge, an accretion centre erupting basalts at its axis and with minimal sedimentary cover[13-15] Possible extensions of recent Icelandic and Jan Mayen plume activity may thus be sought along the Kolbeinsey Ridge

The Kolbeinsey Ridge (66° 30'N–71° 50'N) extends, with a generally NNE trend, for a distance of approximately 550 km from Iceland to the Jan Mayen Fracture Zone and cuts the Icelandic Plateau at depths of less than 1,300 m (Fig 1) The ridge begins as a subdued N–S trending rift valley and corresponding magnetic anomaly which gradually develops on the outer northern Icelandic Shelf The shallow ridge axis surfaces at the islet of Kolbeinsey, 67° 08'N (ref 16) North of Kolbeinsey Islet as far as the Spar Fracture Zone (69°N) the ridge strikes N 28°E and has a step-like structure lacking a central rift valley,

it is similar in morphology to the northern Reykjanes Ridge In addition, this ridge segment also resembles the Reykjanes Ridge in spreading rate, free-air and Bouguer anomalies, and magnetic anomalies[14] Remanent magnetisation values on drilled cores from pillow basalts from this ridge segment obtained by our shipboard spinner magnetometer averaged 0 05 e m u , similar to the average values found for pillow basalts on the Reykjanes Ridge[17] Furthermore, thin sections show phenocryst assemblages of plagioclase–olivine–clinopyroxene (dredges 7–72, 9–72, see Table 1) which are also characteristic for pillow basalts from the Reykjanes Ridge[4,18] Thus, the conclusion[14] that this segment of the Kolbeinsey Ridge seems to be the northern counterpart of the Reykjanes Ridge is probably correct

North of the Spar Fracture Zone, the Kolbeinsey Ridge is offset about 30 km to the east and then takes on the classical mid-oceanic ridge structure with a well developed rift valley trending N 14°E An unnamed fracture zone at 70° 52'N delimits the southern side of Eggvin Bank, a broad seamount transected by the ridge axis From the top of Eggvin Bank, only 50 m below the waves, the ridge gradually deepens towards the Jan Mayen Fracture Zone which reaches depths greater than 2 km

Chemistry of Kolbeinsey Ridge tholeiites

Table 2 presents the concentrations of K_2O, TiO_2, P_2O_5 and Sr in tholeiites from the Kolbeinsey Ridge, these have been plotted against the morphology of the ridge axis (Fig 1) Tholeiites from the ridge axis between 67° and 70° 35'N have similar low contents of the incompatible elements, low levels of K_2O, TiO_2, P_2O_5 and Sr characterise fresh mid-oceanic ridge tholeiites[19-21] In detail, the levels of K_2O, P_2O_5, Sr, and to a lesser extent TiO_2, are generally lower than oceanic ridge tholeiites south of the Charlie Gibbs Fracture Zone[20], and are more comparable to contents of the central and southern Reykjanes Ridge[4,18,22]

Eggvin Bank also consists of tholeiitic basalts but their elemental concentrations deviate sharply from the 'background level' determined for the ridge to the south Figure 1 shows that the concentrations of K_2O, TiO_2, P_2O_5 and Sr rise to a maximum at the upper parts of Eggvin Bank This peak in no way corresponds to the presence of highly evolved or fractionated basalts the $FeO*/MgO$ ratio of Eggvin Bank tholeiites is comparable with the ratio for tholeiites from the adjacent ocean floor to the south Moreover, fractional crystallisation would lead to a similar passive concentration of elements such as K and P which enter early mineral phases in negligible amounts In fact, K_2O concentrations are increased by a factor of seven over ridge values whereas P_2O_5 is only increased twofold The concentration of alkali and volatile elements within a high-level magma chamber[23] of Eggvin Bank is a possibility Alternatively, the lower TiO_2/P_2O_5 ratio of these summit tholeiites
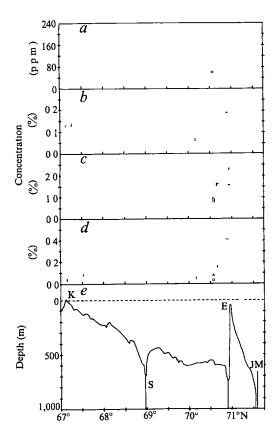
Fig 1 Geochemical (a-d) and topographic (e) profiles along the Kolbemsey Ridge Concentrations of fresh tholeiites a, Sr, b, P₂O₅, c, TiO₂ d, K₂O e K, Kolbemsey Islet[16-21], E, Eggvin Bank, S, Spar Fracture Zone, JM, Jan Mayen Fracture Zone

compared with the adjacent ocean floor tholeiites may indicate a deeper origin for the Eggvin Bank basalts[24]

Interesting comparisons exist between Eggvin Bank and Iceland they are both elevated topographic features, partly bounded by fracture zones and cut by an accretion axis, and significant scatter is found in the concentrations of their incompatible elements

Tholeiites on the southern and northern flanks of Eggvin Bank show concentrations of incompatible elements which are

intermediate between those of the ridge axis and the summit of the seamount The decrease in the TiO₂/P₂O₅ ratio is also apparent in these intermediate samples We have only one sample (1351-1) from the northern flank of Eggvin Bank, recovered from a depth of 400-650 m at the ridge axis It shows no sign of approaching a more alkalic province although true alkali basalts have been recovered from the walls of the Jan Mayen Fracture Zone slightly to the north (unpublished data) It seems that this sample marks a return to ocean floor tholeiite chemistry Schilling[25] has reported, however, a normalised La/Sm ratio greater than 1 0 for a ridge sample from a deeper dredge only 15′ of latitude south of the Jan Mayen Fracture Zone Certainly, our samples do not provide evidence for any southward plume overflow from the Jan Mayen province extending to our most northerly sample, about 30′ of latitude south of the Jan Mayen Fracture Zone Three samples in dredge 1350-4 are also available from just to the south of the fracture zone at 70° 52′N They were recovered from the flank of the ridge axis and exhibit elevated concentrations of K₂O (0 15 to 0 20%) These slightly higher K₂O concentrations could reflect limited degrees of low temperature, seawater alteration and/or more potassic flank volcanism

In conclusion, the chemistry of the most southerly samples recovered from the Kolbemsey Ridge show no indication of approaching the postulated Icelandic mantle plume, the most northerly samples show no chemical similarities to samples from the highly alkalic Jan Mayen province Kolbemsey Ridge crest tholeiites, with the exception of those from Eggvin Bank, show very low concentrations of incompatible elements, reminiscent of the central and southern Reykjanes Ridge Eggvin Bank is considered to be the summit of a tholeiitic seamount sequence with enhanced contents of K₂O, TiO₂, P₂O₅ and Sr

Compositional gradient

We have assembled concentrations of K₂O for tholeiites from the Charlie Gibbs Fracture Zone at about 52° 46′N (Fig 2) through the presumed Iceland plume and along the Kolbemsey Ridge to the Jan Mayen Fracture Zone at about 71° 50′N, a distance of about 2,200 km There is a certain degree of symmetry north and south of Iceland both in the elevation of the ridge axis and also in its structural, geophysical and petrographic character Large sections of this geochemical profile remain unsampled, though some of the critical sections are reasonably well described With the exception of the samples recovered from Eggvin Bank and the Minia Seamount just east of the ridge

Table 1 Dredge stations, depth of recovery and rock description

Dredge no	Latitude and longitude	Depth (m)	Locality	Rock description
D7-72	67° 32 3′N 18° 31 2′W	445-467	Ridge crest	Pillow basalts 10-20% vesicles (≤ 1 5 cm across) 5-10% plagioclase phenocrysts (≤ 3 mm across)+smaller clinopyroxene and olivine crystals
D9-72	67° 58 5′N 18° 23 6′W	576-622	Ridge crest	Devitrified vesicular basalt, 15 % microphenocrysts of plagioclase+ olivine+clinopyroxene
D9-73	70° 10 0′N 15° 15 6′W	1,098-1,281	Rift valley	Large pillow basalt fragments Rare olivine phenocrysts+micropheno-crysts of olivine+plagioclase
D30-73	70° 34 4′N 14° 41 4′W	1,061-1,171	Rift valley	Pillow fragments with hyaloclastic upper surfaces 1-10% phenocrysts of plagioclase (≤ 5 mm across)+rarer olivine
D1350-4†	70° 39 6′N 14° 18 7′W	1,000-1,400	Ridge axis flank	Pillow fragments, < 1 % vesicles Few olivine and plagioclase phenocrysts
D9-73	70° 52 6′N 13° 44 4′W	1,098-1,281	North wall of the fracture zone at junction with ridge axis	Single basalt fragment, < detached pillow tops Basalt fragment has 5 % cavities (≤ 1 cm across)+10 % zoned and resorbed plagioclase phenocrysts+rare clinopyroxene and olivine phenocrysts
D28-73	70° 55 9′N 12° 59 4′W	128	Eggvin Bank	Angular vesicular basalt fragments 5-30 % vesicles (≤ 2 cm across) 10-20 % phenocrysts of plagioclase+olivine±clinopyroxene, all ≤ 3 mm across
D29-73	70° 55 9′N 12° 59 4′W	55-110	Eggvin Bank	Vesicular basalts+tuff fragments Basalts have 10-50 % vesicles (≤ 1 cm across), ∼ 10% amygdales and up to 5% plagioclase clusters± olivine±clinopyroxene
D1351-1†	71° 03 3′N 12° 58 4′W	400-650	Ridge axis	Angular pillow basalt fragment Irregular vesicles (≤ 1 cm across) Few per cent of plagioclase microphenocrysts up to 1 mm across

*Thin section studies, as well as chemical analyses, indicate that the samples reported here are fresh and unaltered
†Samples provided by Dr A Sharaskin, Vernadsky Institute, Moscow, from cruise of RV Akademik Kurchatov, summer 1973

<div style="text-align:center">Table 2 Incompatible element concentrations in tholeiites from the Kolbeinsey Ridge</div>

Dredge no	No of samples	K_2O concentration (%) Average	K_2O concentration (%) Range	TiO_2 concentration (%) Average	TiO_2 concentration (%) Range	P_2O_5 concentration (%) Average	P_2O_5 concentration (%) Range	Sr concentration (p p m) Average	Sr concentration (p p m) Range
Kolbeinsey Islet*	3	0 036	0 03–0 04	0 67	0 66–0 68	0 09	0 06–0 12	80	65–110
D7–72	4	0 076	0 069–0 084	0 88	0 86–0 90	0 09	0 08–0 09	55	55–55
D9–72	1	0 065		0 86		0 08		55	
D7–73	6	0 063	0 041–0.088	1 02	0 76–1 16	0 08	0 06–0 09	65	60–65
D30–73	12	0 047	0 023–0 080	0 72	0 68–0 75	0 10	0 08–0 12	60	55–65
D1350–4	3	0 17	0 15–0 20	1 26	1 24–1 29	0 10	0 09–0 10	70	70–70
D9–73	1	0 19		1 20		0 21		120	
D28–73	5	0 44	0 35–0 56	1 14	0 97–1 27	0 19	0 16–022	145	125–185
D29–73	5	0 44	0 38–0 50	1 63	1 25–1 91	0 21	0 18–0 25	120	95–130
D1351–1	1	0 21		0 91		0 09		115	

* Refs 16 and 21

axis, and D1350-4 from the ridge flank, all materials are from the ridge axis

All samples chosen for study were fresh Thin sections indicated that alteration was limited to slight discoloration of plagioclase and olivine phenocrysts, grains of chlorite, iddingsite and clay minerals were not observed Whole rock water contents did not exceed 0 7% Zonal variation in the concentrations of K_2O was observed in several basalt pillows For example, samples from D30–73 show their highest K_2O concentrations in the intermediate variolitic zone (0 078–0 079%), compared with the glassy margins (0 036–0 044%) and cores (0 027%)

There is some correlation between the topographic profile and the K_2O concentrations in the tholeiites Three main divisions of the K_2O profile may be distinguished First, the great expanse of ocean floor, about 55% of the profile, where K_2O values range from about 0.03 to 0 11% K_2O, second, the Icelandic geochemical anomaly, where the uncertain line of best fit rises to a maximum of about 0 29%, and where the scatter of potash concentrations is at a maximum The seamounts of Minia and Eggvin both at the extreme limits of this profile and close to major fracture zones also represent scattered high-concentration potash zones, although much narrower than Iceland Lastly, there is a mixed belt for the northern Reykjanes Ridge, where K_2O concentrations rise from oceanic to Icelandic levels There is some indication of a 'plateau' in the trend, corresponding to the southern insular shelf of Iceland Certainly, the K_2O contents of tholeiites reported from this shelf show no clear increase towards Iceland, although extending over 20' of latitude[26]. From the available evidence it can be seen that an extensive mixed zone is not present (Fig 2) on the northern shelf of Iceland This shelf is cut by the Tjornes Fracture Zone which offsets the spreading axis by about 100 km (ref 27) and may act as a barrier to any northward flow of the asthenosphere[25]
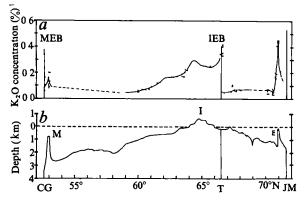
Fig. 2 K_2O concentration (*a*) and topographic (*b*) profiles along the mid-oceanic ridge between Charlie Gibbs (CG) and Jan Mayen (JM) Fracture Zones *a*, K_2O contents from fresh tholeiites[4,16,18,21,22,26,36], Major element boundary (MEB) from Campsie *et al*[18] and incompatible element boundary (IEB) from both Schilling *et al*[25] and this study *b*, M, Minia Seamount, E, Eggvin Bank, I, Iceland, T, Tjornes Fracture Zone

Of particular interest is the fact that the low K concentrations of the ocean floor tholeiites to the north and south of Iceland are shared by Iceland 'plume' theoliites The average K_2O concentrations of Icelandic tholeiites, both from the Tertiary plateau and the neovolcanic zones, are about 0 25% (unpublished compilation), and although this value is higher than that of the adjacent ridge tholeiites, it lies within the worldwide range of K_2O concentrations for fresh, ocean ridge tholeiites[20,21]

In contrast to Iceland and its environs, K_2O concentrations for the Galapagos plume tholeiites average about 0 36%, and the surrounding ocean floor tholeiites average close to 0 14% (refs 28 and 29) Thus, K_2O concentrations for tholeiites of the Icelandic region, regardless of their genesis, are about 70% of those for tholeiites of the Galapagos plume and its environs It follows that the geochemistry of plume magmas is to some extent integrated into the geochemical pattern of a broad region, and does not simply represent an independent magma type derived from independent deeper regions of the mantle, as implied by Morgan[6] Perhaps a fairly constant factor, additive or multiplied, will convert the K_2O concentrations of the plume to normal regional concentrations

Relationship between the plume and surrounding crust

There is one possibility whereby the Iceland 'plume' may be interrelated with the surrounding ocean floor The Iceland area may have begun at a 'weak spot' rather than at a 'hot spot' Such 'weak spots' may be located close to triple junctions or perhaps at the intersection of spreading axes and pre-existing tectonic lineaments, such as that delineated by the trend of the Faeroe–Iceland–Greenland ridge

Localised decompression would lead to more extensive melting and increased basaltic discharge Basalts generated at this focus of decompression would be equilibrated at shallower levels than normal, explaining the generally higher FeO*/MgO ratios of the basalts and also their lower eruptive temperatures[30] Further supplies of basaltic material would be sucked into this lower pressure region through the low velocity zone, preferentially bringing the more mobile incompatible elements with them[18] This transfer of material may lead to subsidence of the oceanic crust There may be little or no basalt replenishment because some fracture zones in this region may mark deep barriers to asthenospheric flow Therefore, any basalts generated in the regions of greatest subsidence will have low concentrations of incompatible elements and higher concentrations of refractory MgO, giving lower FeO*/MgO values The amounts of K, P, and so on transferred to the decompression zone may depend on the regional geochemical levels, and on the rate and degree of flow Excess volumes of basalts may be discharged away from Iceland at shallow crustal levels and may be mixed with normal oceanic tholeiites within elongated magma chambers[4] The available evidence suggests that while the Iceland landmass is rising[3], the seafloor to the north has subsided[31-34]

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Some evidence of chronology and palaeoecology of Sterkfontein, Swartkrans and Kromdraai from the fossil Bovidae

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Bovid fossil remains suggest a tentative chronology for the seven cave assemblages, from Sterkfontein, Swartkrans and Kromdraai, from which they are derived They also suggest that, during the almost 2 Myr of South African hominid and faunal Pleistocene history encompassed by this succession of assemblages, certain changes in vegetation cover and cave accumulation patterns may have occurred in the Sterkfontein Valley

THE cranial bovid material derives from the following subdivisions, or site units, of Sterkfontein, Swartkrans and Kromdraai, all situated in the Sterkfontein Valley (see Fig 37 in ref 1) near Krugersdorp, South Africa STS, Sterkfontein type locality, SE, West Pit of the Sterkfontein extension locality, D16, Sterkfontein rubble dump 16 , SKa, Swartkrans assemblage from primary[2] (formerly pink[1]) breccia, SKb, Swartkrans assemblage from (1) secondary[2] (formerly brown[1]) breccia, and from (2) fills of channels forming at a relatively late stage through both primary and secondary breccias, KA, Kromdraai faunal site, KB, Kromdraai australopithecine site A brief summary of some currently available ideas on the hominid and cultural associations of these site units is shown in Fig 2 and the bovid species identified at these site units are shown in Table 1

Chronology

Much of the bovid material was found to correspond taxonomically to a greater or lesser extent with material from other sites (discussed in ref 3) Figure 1 shows the temporal sequence of the site units, and its possible correspondence with other African sites and with the absolute time scale, as suggested by these bovid data The most notable changes from previously accepted

chronological evaluations are as follows It is clear that Swartkrans fossils include a substantial component that may be considerably later than the assemblage associated with australopithecines, and that Swartkrans cannot, as in the past, be considered as one unit in studies of this kind The present bovid data also suggest that KA, KB and SE should not be included in the Swartkrans span, as has been variously done in the past, but that they may belong to the succeeding Cornelia span Note that the present study was uninformative as to how far back in time STS and Makapansgat Limeworks may have extended[3] These positions in Fig 1 represent upper limits only

Vegetation cover and climate

It is assumed here, as previously[8], that the Alcelaphini and Antilopini are as a whole, among bovid tribes, indicative of open plains and grassland environments The validity of such an assumption in this particular study, especially its application to unusual fossil forms like *Makapania*, has been discussed[3] The fact that the percentages in Fig 2 never fall below 51 % seems to indicate that the past environments, corresponding to these assemblages, as a group fall distinctly into the vegetationally more 'open' part of the spectrum of known habitats Seen from a broad perspective, therefore, these bovid data may corroborate the view[11] that each of the breccias in question basically represents colluvial sediments compatible only with an incomplete mat of vegetation Figure 2, however, suggests differences within this overall environmental characterisation The most notable difference lies between STS and the group formed by SKa, KA, SE and, less certainly, KB (the difference in percentages of STS and SKa is statistically significant[3]) It cannot be suggested that the smooth curve in Fig 2 mirrors an equally smooth, simple environmental trend during the Pleistocene in the Sterkfontein Valley Some quite substantial fluctuations may not be included in this small sampling of the whole period Figure 2, however, may broadly represent an overall trend in the area, and probably further afield It is likely that during most of the Middle Pleisto-

Table 1 Minimum numbers of individuals in bovid species at each of site units STS, SKa, KA, SE, KB, SKb and D16 Taxonomy as in ref 3

	STS	SKa	KA	SE	KB	SKb	D16
Tribe Alcelaphini							
Gp Ia *Damaliscus* cf *dorcas*				3		9	11
Gp Ib *D* sp 2 (*niro*?)	1			4		14	5
Gp Ic *D* sp 1 or *Parmularius* sp	7	2	32	1			
Gp II Medium-sized alcelaphines	7	24	12	5		6	1
Gp III cf *Connochaetes taurinus* lineage	1	16	5	1	3	2	1
Gp IV cf *Megalotragus* sp	1	5	2				
Tribe Hippotragini							
Hippotragus cf *niger*						9	4
H cf *equinus*	2		1				
Hippotragini(?)	8	1	1	1			
Tribe Reduncini							
cf *Kobus ellipsiprymnus*						2	
Redunca cf *arundinum*	1	1	1				
Tribe Peleini							
Pelea cf *capreolus*		2	3			7	3
Tribe Antilopini							
Antidorcas cf *australis* and *A* cf *marsupialis*						10	2
A cf *recki*	3	13	13	3	2		
A bondi	1	7	1	2	2	63	7
cf *Gazella vanhoepeni*	1	11					
Gazella sp					1		
Gen et sp indet (Antilopini or Neotragini)		3					
Tribe Neotragini							
Oreotragus cf *oreotragus*					2		
O cf *major*		1		1	1		
Raphicerus cf *campestris*					5	2	
cf *Raphicerus* sp			1		1		
Ourebia cf *ourebi*					3	3	
Tribe Bovini							
Syncerus sp	1	4	2				
Tribe Tragelaphini							
Tragelaphus cf *scriptus*		2	1		2	2	
T cf *strepsiceros*		5	5		3		
T sp aff *angasi*	1	1					
Taurotragus cf *oryx*			2	1	1	1	
Tribe Ovibovini							
cf *Makapania* sp		4					
Makapania cf *broomi*	8		1				
Incertae sedis					1		
Totals	43	102	87	23	9	140	42

Minimum numbers of individuals, rather than the basic fossil numbers available in ref 3, are used This is in accordance with general practice in work on other Transvaal cave fauna (see also ref 4)

cene an open grassland environment predominated The onset of this open phase (in the broad sense and allowing for oscillations) succeeded a period of greater bush cover, although open country had also been prevalent during this period, as represented by STS and probably the Makapansgat Limeworks It has been mentioned previously[8] that either rainfall and/or temperature changes are likely to be responsible for vegetation cover changes such as that inferred between STS and succeeding Sterkfontein Valley phases, and that an explanation of the present bovid data invoking rainfall even minimally would seem to contradict Brain's findings that a general increase in rainfall, with minor fluctuations, occurred in the Sterkfontein Valley from the time of STS onwards

There is agreement from several sources[12,13] that a general faunal change took place in South Africa between STS and SKa times The bovid data suggest that an environmental change may have been responsible for the faunal change, at least in the Sterkfontein Valley It is thus not entirely idle speculation to consider whether the change from gracile australopithecines at STS and Makapansgat Limeworks to robust forms at SKa and KB may be environmentally correlated Published opinions on fossil hominid taxonomy range from those that place all Transvaal australopithecines into one species, possibly in chronosubspecies[14], to those that see them in separate species[15] or separate genera[16] If the latter view were taken one could note

that the basic premises of Robinson's[16] dietary hypothesis would fit an open grassland–robust correlation very well, although the last step (based on ref 1) of his argument would not, that is, seeing the 'crushing, grinding' robust vegetarian in a (slightly) wetter and more luxuriant environment than the gracile omnivore If one views the SKa and KB australopithecines as differently adapted to their gracile counterparts, perhaps one should consider whether their musculature was so massive and, the molars proportionally so large, because their 'vegetables' were of the tough grassland type The long-adapted Phase 1 hominid, or 'small object' vegetarian of Jolly's 'seed-eaters' hypothesis[17] fits well in this respect Of course, if these robust and gracile forms were considered to be similarly adapted[14], with the often cited skull and dentition differences merely a consequence of overall size difference, the only admissible correlation would be between vegetationally more open (and drier and/or colder) environments and larger body size

Is it a coincidence that an East African proliferation of bovids adapted to open country, apparently occurring during or above member G at Omo (A W Gentry, unpublished) and in the *M andrewsi* zone at East Rudolf (J M Harris, unpublished), seems to coincide temporally with the analogous South African development? It could be possible that such a change in faunal tribal representation, mirroring a change in environmental exploitation, marked the boundary between a Sterkfontein span (or similar term) and a succeeding span not only in the Sterkfontein Valley, but elsewhere in Africa as well

Possible accumulation patterns

Some tentative suggestions as to the possible predominant causes of accumulation of these bovid assemblages are shown in Fig 3. To evaluate such aspects in this particular case, where only adult-juvenile and weight data of bovids from a series of cave accumulations are available, the following model was set up
Stage 1 bovids are killed by carnivore or hominid predators, (hominid hunters are included as a subset of the set of predators) in the vicinity of the cave, or die a natural death in or near the cave
Stage 2 remains of dead animals are brought into the cave eventually to constitute one or the other of the following
(a) Primary assemblage brought into the cave, which serves as a lair or shelter, by the primary predator(s), unlikely to contain a low percentage of juveniles, and if one predatory pattern predominates, most individuals should fall into a restricted body weight range
(b) Secondary assemblage brought into the cave by scavengers (for example, hyaena, hominid) and/or collectors (for example, porcupine, hominid), likely to contain a low percentage of juveniles, and the body weight distribution is likely to have a high variance
A fuller discussion of this suggested scheme, and of the validity of the implied characteristics of primary and secondary assemblages, is contained in ref 3 Most of the data in Fig 3 do not lend themselves to a statistical approach, and *what follows is a number of suggestions as to possible dominant accumulation patterns*
Juveniles of the smaller species are often eaten totally, or almost totally, by predators[18] and consequently have less chance of being preserved in fossil accumulations One can therefore expect fossil juvenile-adult ratios to be heavily biased towards adults With this consideration in mind juvenile percentages in STS, KA and D16 particularly, and in SKa, KB and SKb (Fig 3a), seem high in comparison with those recorded in live populations[19,20,21] In fact it looks as if juveniles might have been selected in preference to adults in these cases, and it is likely that they represent primary, or predominantly primary assemblages SE clearly differs from the other site units in this respect
STS. The most frequently occurring species are of medium to large size—weight class I is entirely absent The exceptionally high average weight (Fig 3c) indicates predators that were
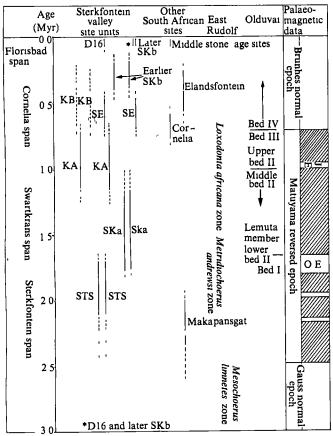
Fig 1 The bovid data suggest chronological placement of the Sterkfontein Valley site units somewhere along the vertical lines drawn Palaeomagnetic and Olduvai data approximated from ref 5, East Rudolf faunal zones from refs 6 and 7 JE, Jaramillo Event, OE, Olduvai Event

specialised on large prey It cannot be ruled out that *Australopithecus* contributed in the role of scavenger, but his main 'contribution' is likely to have been as carnivore prey STS carnivore remains include *Megantereon*, a true sabre-tooth cat, *Dinofelis*, a false sabre-tooth cat, and a leopard[22,23] As individuals of the size preferred as prey by modern leopards[18] are in the minority, it seems likely that the major predators were the sabre-tooths Ewer[24] has pointed out that, as a result of highly efficient carnassial shear, these carnivores were well adapted to the slicing of meat The premolars, however, were so reduced that the animals could only have crushed the smaller bones, leaving the niche for bone-crushing specialists, like hyaenids, wide open Brain[25] further elaborated on this idea "Regrettably little is known about the behaviour of South
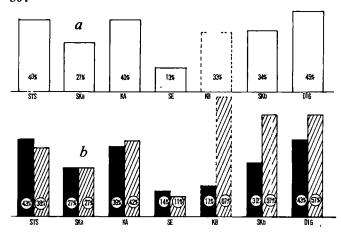
African sabre-tooths and it is uncertain whether they were dominant to the associated hyaenas or whether they were forced to retreat with their prey into the seclusion of caves or trees"[17] During much of the accumulation period the STS cave may have been such a sabre-tooth lair Whole kills, or parts of larger kills may have been brought to the cave for peaceful consumption by the predator and its brood

SKa and KA. Brain[25] has given convincing reasons why the predominant contribution to the SKa assemblage should have come from leopards In comparing remains from KA and KB (C K Brain, personal communication) he has noted the following Although the more complete specimens in the former assemblage suggest that KA may have been a carnivore lair, the extremely fragmented KB bone remains may represent food remains of hominid hunters The bovid assemblages from SKa and KA seem to support these ideas In both cases the majority of individuals include high percentages of juveniles and fall into a restricted medium weight range, suggesting dominant predators of a somewhat smaller prey adaptation than those at STS (in the case of KA possibly the large lion-like felid present in that assemblage) As at STS the percentages of juveniles do not increase in the larger weight categories (Fig 3b) In both cases adult bovids in the largest weight class, as well as sabre-tooth remains, are present, suggesting that these predators may have played a secondary role

KB, SKb, D16. The last three site units in Fig 3 have several features in common Like the three earlier carnivore assemblages they have high overall juvenile percentages, although they diverge sharply from STS, SKa and KA in Fig 3b and c All three later assemblages are strongly dominated by species of low weight In each case the few large-sized individuals include a distinctly higher percentage of juveniles than do the lower weight classes The pattern is again strongly suggestive of predation, this time by a predator specialised on small prey The three site units have more in common They have been placed last in the chronological sequence on taxonomic grounds The presence of both *Homo* and stone tools is either known, or inferred for reasons independent of their similarity in Fig 3, in all three site units The obvious conclusion is that KB, SKb and D16 each represent (predominantly, if not entirely) the food remains of hominid hunters Note that while scavenging probably did play a part, Fig 3a–c are unanimous in stressing an over-riding hunting pattern

SE. The presence at this site unit of numerous stone tools suggests that the Sterkfontein cave during SE times may have been an occupation site of hominids Yet the pattern at SE (Fig 3) is quite different from those presented by the inferred hominid hunting remains from KB, SKb and D16 In fact, its markedly low juvenile percentage and the broad, platykurtic distribution of only 23 individuals throughout all weight classes, suggest that SE represents the only markedly non-primary

Fig 2 The percentage constituted by the added minimum numbers of alcelaphine and antilopine individuals of the total minimum number of bovid individuals, per site unit, is suggestive of the degree of bush cover during deposition (as crudely represented by the small trees) Solid line hominid figures express widely accepted taxonomic evaluations, dotted lines signify in the cases of KB and D16 that the presence of *Homo* is only inferred, in the case of SE that uncertainty exists as to which hominid(s) is (are) present, but that *Homo* may be included[9] Cultural associations of SKa and SE as in ref 10, of KB as in ref 1, of SKb according to Brain (unpublished), of D16 inferred Percentage cover eight trees, 51–55%, seven, 56–60% and so on, one, 86–90%, none, 91–100%
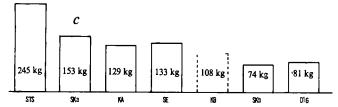
Fig 3 Changes in average body weight and in juvenile proportions from site unit to site unit Weight classes I–IV of Bovidae as defined in ref 4, all data for *a–c* from ref 3 *a*, Percentage juveniles of total minimum number of individuals in each site unit *b*, Percentage juveniles present in combined weight classes I and II (solid columns, <80 kg per individual) on the one hand, and III and IV (hatched columns, >80 kg per individual) on the other, in each site unit *c*, Estimated average weight (kg) per individual, in each site unit

assemblage among those considered in this study An hypothesis that the SE toolmaker was a scavenger would fit the available data very well If the extensively fragmented SE bovid remains were indeed the discards of meals scavenged from carnivore kills, one would not expect a high percentage of juveniles, nor an increased sampling of juveniles in higher weight categories (Fig 3*b*), although the average weight of what is consumed may still be as high as that of a primary assemblage of carnivore remains (Fig 3*c*)

The pattern presented by the bovid remains is consistent with their chronological succession In the earlier three assemblages

there is strong evidence of carnivore occupation of the Krugers-dorp caves, and such parameters as those used here agree consistently from site unit to site unit Thereafter the only evidence available here indicates (at least predominant) hominid occupation of caves in the same places that were previously dominated by carnivores Were carnivores dominant to earlier hominids, but ousted by their more advanced descendants, in the competitive search for shelter in the Sterkfontein Valley? Certainly the site units here investigated can be divided into an earlier carnivore occupation phase and a later hominid occupation phase Within the latter, the hominid quest for animal protein may have first led to scavenging, and only later to hunting If the present interpretation of bovid remains is correct, it would support the conclusion of a number of authors that tools were being fashioned before attainment of the hunting adaptation SE tools, serving to procure vegetable foods and to prepare scavenged meat, may have been the hominid pre-adaptation for the hunting which is evident in the later site units

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Structural invariants in protein folding

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An analysis of 15 protein structures indicates First, the loss of accessible surface area by monomeric proteins on folding—proportional to hydrophobic energy—is a simple function of molecular weight, second, the proportion of polar groups forming intramolecular hydrogen bonds is constant, and third, protein interiors are closely packed, each residue occupying the same volume as it does in crystals of amino acids

THE aesthetic shock of the first protein structures is expressed in Kendrew's contemporary description of myoglobin[1] as "almost nothing but a complicated set of rods [of poly-

peptide] sometimes going straight for a distance then turning a corner and going off in a new direction much more complicated and irregular than most of the early theories of the structure of proteins had suggested"

Although structural similarities between proteins of different sequence but similar functions have become apparent, the image of protein structure as a disordered and complex assembly of pieces of secondary structure has continued to the present day

At the same time as the first protein structures became known a simple qualitative theory for the energies responsible for maintaining protein structure was described, principally

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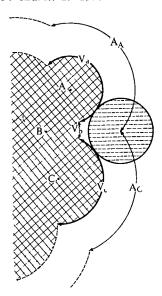
Fig 1 Two-dimensional illustrations of the concept of accessible surface area—the area over which the centre of a water molecule can be placed while retaining van der Waals' contact with that protein atom and not penetrating any other atom Three atoms, A, B, and C have the van der Waals' envelopes V_A, V_B and V_C which define the surface of the protein Atoms A and C have the accessible surface represented by the arcs A_A and A_C, A and C sterically prevent any contact between water molecule shown and atom B which therefore has no accessible surface

by Kauzmann[2] Essentially, this stated that when a protein folds the gain in hydrophobic energy, as a result of the reduction in the number of non-polar contacts with water, compensates for the loss of chain configurational entropy, and that polar groups on the interior of the protein form hydrogen bonds[3]

Here I show that, if in place of chain topology, structural descriptions are used which are directly related to energy terms—accessible surface areas, hydrogen bonds and residue volumes—there are simple equations or expressions which quite accurately describe protein structures

The atomic coordinates of 15 proteins were used lysozyme, α-chymotrypsin, insulin, rubredoxin, porcine trypsin inhibitor (PTI), high potential iron protein (HIPIP), calcium binding protein (CBP), ribonuclease S, staphylococcal nuclease, papain, concanavalin A (con A), subtilisin, thermolysin, carboxypeptidase A and lactate dehydrogenase (LDH) The

atomic coordinates of the first two proteins were energy refined by Levitt[4,5] and I carried out a preliminary energy refinement of the coordinates of the last 10

The structures of haem proteins are not described by some of the relationships derived below and will be dealt with elsewhere

Accessible surface area of proteins

The term "accessible surface area" has been introduced by Lee and Richards[6] to describe the area over which contact between protein atoms and water molecules can occur For a particular atom, this term is defined as the area over which the centre of a water molecule can be placed while retaining van der Waals' contact with that atom and not penetrating any other protein atom (Fig 1) Atomic accessible surface areas were calculated by Lee and Richards[6] for lysozyme, ribonuclease S and myoglobin, and by Shrake and Rupley[7] for lysozyme and insulin It was later shown that in proteins the hydrophobic free energies are directly related to the accessible surface area of both polar and non-polar groups[8] Here I show that the hydrophobic energy of proteins—proportional to the loss of accessible surface area that occurs on folding—is related to its molecular weight by a simple equation

In the calculations described below the following abbreviations are used

A_S, The accessible surface area of the folded native protein

A_T, The accessible surface area the protein would have if it unfolded and assumed an extended conformation with the side chains *trans*

A_B, The accessible surface area that is buried when the protein folds, equal to $A_T - A_S$

F_{AS}, The proportion of the proteins' accessible surface that is buried when it folds equal to A_B/A_T

Accessible surface areas were found using the method described by Lee and Richards[6] and a computer program written by Levitt (personal communication) Hydrogen atoms are not considered individually but included in the van der Waals' radii used for non-hydrogen atoms The van der Waals' radii used, oxygen 1 40 Å, trigonal nitrogen 1 65 Å, tetrahedral nitrogen 1 50 Å, tetrahedral carbon 1 87 Å, trigonal carbon 1 76 Å, sulphur 1 85 Å and water 1 40 Å, were based on the intermolecular contact distances observed between non-hydrogen atoms in the accurate crystal structure analysis of amino acids (Koetzle, personal communication) Accessible surface areas of individual residues, R, were calculated using the peptide Gly-R-Gly which has an extended β conformation ($\Phi = -140°$, $\Psi = 135°$) and the side chains fully extended

Table 1 Accessible surface area and proportion of polar groups forming intramolecular hydrogen bonds in 15 proteins
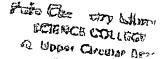
Protein	Molecular weight	A_T	A_S	A_B	Calculated A_B	F_{AS}	F_{HB}
Insulin*	5,787	8,380	3,440	4,940	(5,230)	0 59	0 37
Rubredoxin†	6,108	8,670	3,320	5,350	5,530	0 62	0 36
PTI	6,158	9 440	3,700	5,740	5,580	0 61	0 36
HIPIP	8,976	13,380	4,600	8,780	8,330	0 66	0 46
CBP	11,489	16,740	5,740	11,000	10,890	0 66	0 52
Ribonuclease S	13,690	19,840	6,550	13,290	13,210	0 67	0 42
Lysozyme	14,814	20,560	6,480	14,080	13,880	0 68	0 48
Staphylococcal nuclease‡	15,973	23,800	7,840	15,960	15,690	0 67	0 48
Papain	23,423	33,850	9,210	24,640	24,360	0 73	0 42
α–Chymotrypsinφ	24,608	35,900	10,130	25,770	25,820	0 72	0 44
Con A*	25,578	37,030	10,230	26,800	(27,030)	0 72	0 40
Subtilisin	27,534	40,090	9,790	30,300	29,510	0 76	0 51
Thermolysin	34,334	49,410	11,570	37,840	38,600	0 77	0 49
Carboxypeptidase	34,409	49,870	11,260	38,610	38,710	0 77	0 50
LDH*	36,470	53,080	17,060	36,020	(41,610)	0 68	0 42

Areas are expressed in $Å^2$ Calculated A_B = the value of A_B given by equation (3) F_{AS} = the proportion of the protein's accessible surface area that is buried when it folds equal to A_B/A_T F_{HB} = the proportion of protein polar groups forming main chain–main chain or side chain–main chain hydrogen bonds
  *Calculation carried out on an isolated monomer
  †Molecular weight of the sequence found by the X-ray analysis (ref 23)
  ‡Calculations do not include the terminal residues 143–149 which are not seen in the Fourier rays
  φCalculations do not include residues 10–16 which are not seen in the Fourier map
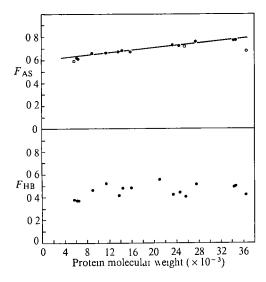
Fig 2 upper, F_{AS}, the proportion of the potential accessible surface area a protein buries when it folds, and lower, F_{HB}, the proportion of protein polar groups forming one or more main chain–main chain or main chain side chain hydrogen bonds, plotted against protein molecular weight The F_{AS} values for oligomeric proteins are represented by open circles

($\chi_1 = -120°$, $\chi_{n+1} = 180°$) The values of residue accessible surface areas were similar to those found by Shrake and Rupley[7], though they used slightly different van der Waals' radii and averaged over a number of different residue conformations

In Table 1, the values for A_T, A_S and A_B are listed for 15 proteins A_T values were found by summing the residue accessible surface areas for each residue present in the protein

As might be expected, A_T is proportional to molecular weight (M) The expression

$$A_T = 1\ 44M \tag{1}$$

gives values in $Å^2$ within 1 % for 12 of the proteins considered and within 3 % for the rest In Fig 2a, the value of $F_{AS} = (A_B/A_T)$ is plotted against molecular weight for each protein The points on this graph can be divided into two sorts the oligomeric proteins, insulin, concanavalin A and lactate dehydrogenase,

and the rest, which are monomeric proteins with between 54 and 316 residues A least squares treatment shows that for the monomeric proteins F_{AS} is linearly related to molecular weight by the equation

$$F_{AS} = 0\ 595 + 0\ 536M \times 10^{-5} \tag{2}$$

The protein accessible surface area which is buried on folding, A_B, is $A_T \times F_{AS}$ Therefore,

$$A_B = 0\ 859M + 0\ 774M^2 \times 10^{-5} \tag{3}$$

This equation gives values for A_B in $Å^2$ to within at least 3 % of those observed (Table 1) for all the monomeric proteins Nozaki and Tanford[9] calculated, from the free energy of transfer of amino acids from water to ethanol or dioxane, the hydrophobicity values of 10 side chains, and if, in the manner described previously[8], these values are equated with the side chain accessible surface areas calculated here, it is found that for each $Å^2$ removed from contact with water there is a free energy gain of 20 calorie (the value given in ref 8 using the side chain accessible surface areas of Lee and Richards, was 24 calorie) If the transfer is from water to hydrocarbon solvents, instead of to ethanol, free energy values are about 20% higher[10] Therefore taking the hydrophobicity of amino acid residues to be 24 calorie per $Å^2$ the hydrophobic contribution to the free energy of folding, E_{HP}, in calories is (from equation (3))

$$E_{HP} = 21M + 19M^2 \times 10^{-5} \tag{4}$$

The application of equation (2) to proteins larger than those considered here is obviously limited The little information so far available (for example, refs 11–14) indicates that the structures of large globular proteins consist of quite distinct domains of a size similar to the molecules considered here and that these domains can by themselves retain a functional structure This suggests that for large globular proteins F_{AS} is essentially constant

There are three oligomeric proteins in the sample considered here insulin, con A, and lactate dehydrogenase The observed values of F_{AS} for insulin and con A, 0 59 and 0 72 respectively, are smaller, though not significantly, than the values calculated

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Table 2 Volume occupied by residues in the interior of 9 proteins

Residue	Total no in the proteins	No buried*	Average volume (V_R) of buried residue ($Å^3$)	S D of V_R($Å^3$)	†Residue crystal volume equal to amino acid volume less 11 1$Å^3$
Val	163	91	141 7	8 4	143 4
Ala	183	71	91 5	6 7	96 6
Ile	106	69	168 8	9 8	169 7
Gly	160	60	66 4	4 7	66 5
Leu	138	57	167 9	10 2	—
Ser	190	46	99 1	7 4	102 2
Thr	128	32	122 1	6 7	124 3
Phe	60	29	203 4	10 3	—
Asp	117	17	124 5	7 7	122 0
Cys	34	16	105 6	6 0	108 7
Pro	67	16	129 3	7 3	124 4
Met	28	14	170 8	8 9	176 1
Tyr	98	13	203 6	9 6	201 7
Glu	65	13	155 1	11 4	143 9
Asn	116	12	135 2	10 1	—
Trp	39	9	237 6	13 6	—
His	43	8	167 3	7 4	166 3
Lys	119	5	171 3	6 8	—
Gln	80	5	161 1	13 0	148 0
Cyh	10	4	117 7	4 9	123 1
Arg	63	0	—	—	—

The nine proteins are CBP, ribonuclease S, lysozyme, papain, α–chymotrypsin, subtilisin, carboxypeptidase, thermolysin and LDH
*A residue is defined as buried if 5 % or less of its potential accessible surface area is available to solvent contact
†11 1 Å is the volume lost by an amino acid on becoming a residue The value used here was found by comparing the crystal volumes of glycine and glycylglycine, and is identical to the value in Cohn and Edsall[22]—11 0$Å^3$—determined by solution studies No accurate unit cell dimensions were found for Leu, Phe, Asn, Trp and Lys
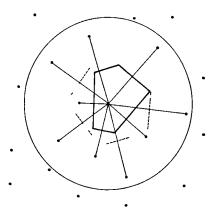
Fig 3 Two-dimensional illustration of Richards' method of finding the volume occupied by an atom in a protein Vectors are made from the atom to all neighbours within a radius of 6 5 Å Planes perpendicular to each of these vectors are then constructed at a point related to the van der Waals' radii of the two atoms forming the vector The smallest polyhedron so constructed—the Voronoi polyhedron—is the volume occupied by the atom (see refs 19 and 20)

from equation (3), 0 63 and 0 73 The observed F_{AS} value for LDH, 0 68, however, is very significantly smaller than the calculated value, 0 79 Subunit contacts involve large hydrophobic free energies[9], and so these observed F_{AS} values suggest, the subunit contacts formed in oligomeric proteins may allow the subunits to have a more open structure than that found for monomeric proteins of the same size

Intramolecular hydrogen bonds in proteins

The free energy of intramolecular protein hydrogen bonds as compared with those between water and protein is unknown Experimental values for the difference in enthalpy are small[15,16] We could expect the entropy contribution to favour protein–protein hydrogen bonding as this would release bound water molecules (as occurs on the melting of ice where each molecule gains 18 e u)

Protein hydrogen bonds were found by using a computer program (Levitt, personal communication) which, given a list of non-hydrogen coordinates, adds hydrogens in positions fixed by the known stereochemistry of H–X–W bond angles and lengths, and then prints out Z–Y   H–X arrangements where Y is a hydrogen bond acceptor, H–X a donor and the geometry is satisfactory for hydrogen bond formation a hydrogen bond was accepted if the distance Y   X was 3 5 Å or less, the angle Z–Y   X more than 90°, and the angle Y   H–X more than 150°

Hydrogen bonds were listed in this way for the 15 proteins In Fig 2b the proportion of the polar groups that were found to form one or more main chain–main chain or main chain–side chain hydrogen bonds, F_{HB}, is plotted against the protein's molecular weight The precise absolute values for F_{HB} are not significant as they depend on the geometrical criteria used to determine hydrogen bonds The differences between F_{HB} values for proteins of molecular weight greater than 8,900 are not significant since the number of polar groups which correctly form hydrogen bonds depends on the quality of Fourier map and the subjection of the initial coordinates to Diamond model building, and real space, difference Fourier and energy refinement[5] This is particularly true of the few groups forming side chain–side chain hydrogen bonds For this reason and because many of them have a transient existence[17,18], they were not included

From Fig 2b, it is unambiguously clear that, for those proteins considered here that have a molecular weight over 8,900, the proportion of polar groups forming intramolecular hydrogen bonds is essentially constant and close to 0 5

For most globular proteins the number of polar groups present is proportional to molecular weight of the 15 considered here

$M/38$ 8 gives the number of polar groups to within 3 % except for lysozyme (4 %) and ribonuclease (5 %) This implies that to a good approximation the contribution of hydrogen bonds to the free energy of protein folding is simply proportional to molecular weight

Volume occupied by residues in the interior of proteins

Globular proteins are distinguished from fibrous proteins and other two-dimensional biological structures by the ability of the pieces of secondary structure from which they are formed to associate and give a stable three-dimensional structure I shall discuss briefly the nature of the molecular interactions that occur between pieces of secondary structure

The problem can be put in terms of the question how well do pieces of secondary structure recognise each other? The quantitative answer can be found by calculating the volumes occupied by residues in the protein interior Large residue volumes (low density) would imply that the recognition was poor and the association similar to that which occurs in micelles, while a small volume (high density) could mean that the recognition is precise and similar to that which occurs in crystalline solids Residue volumes are directly related to packing energies and conformational entropies

The calculation of residue volumes is part of the larger problem of protein density and the most rigorous approach to this is undoubtedly that of Richards[19] who adopted the approach used by Bernal and Finney[20] to describe the structure of liquids, and introduced the use of Voronoi polyhedra to calculate the volumes occupied by protein atoms The volume occupied by a protein atom is found, by first constructing vectors to all neighbouring atoms, second, at a point along each vector related to the van der Waals' radii constructing a perpendicular plane, and third, finding the smallest polyhedron described by these planes (Fig 3) The method and its implementation is fully described in refs 19 and 20 By its application Richards showed the interior of lysozyme and ribonuclease to have a packing density of 0 75, similar to that of small organic crystals

I have calculated the volumes occupied by residues buried in the interior of the calcium binding protein, ribonuclease S, lysozyme, subtilisin, α-chymotrypsin, carboxypeptidase A, papain, thermolysin and lactate dehydrogenase As a working definition, proteins were taken to be buried if less than 5 % of their potential accessible surface was available to solvent contact Atomic volumes were found by using the B version of Richards' computer program[19] and residue volumes found by summing the value calculated for each atom The results are summarised in Table 2 which also lists the volumes which residues occupy in crystals of amino acids

Richards observed that protein interiors are close packed[19] From Table 2 this observation can be extended to state that, in general, the volume occupied by a particular residue in the interior of a protein is constant (the standard deviations are ~6 %), and that it is the same as that occupied by the residue part of the amino acid in its crystal form Thus the "oil drop" model of protein structure with its implication of the micelle-like association of pieces of secondary structure is incorrect, while the description of proteins as "molecular crystals"[21] is accurate The close packing of protein interiors prevents water molecules being trapped in non-polar cavities, maximises the packing energy and implies that the stable association of pieces of protein secondary structure depends on the existence of interfaces that can close pack and are large enough in area to give sufficient hydrophobic energy This can probably be generalised to apply to recognition processes that occur between other kinds of biological molecules

Of the buried residues listed in Table 2 those with non-polar side chains (Val, Ala, Ile, Leu, Gly, Phe and Pro) make up 67 % by number (and 66 % by volume) of the total while those with polar side chains make up 33 % Experimental solution studies indicate that as long as unchanged polar groups retain their

hydrogen bonds their hydrophobicity is similar to that of non-polar groups[8], and, indeed, Lee and Richards found that when ribonuclease S, lysozyme and myoglobin go from an extended conformation to the native structure the reduction that occurs in the accessible surface area of polar and non-polar groups is the same[6] But the necessity for, and geometrical requirements of, hydrogen bond formation by interior polar side chains severely limits the possibilities of formation of close packed molecules by pieces of secondary structure Twenty-six per cent of the serine and threonine residues are buried but only 10% of the asparagines and 6% of the glutamines, both of which have two side chain polar groups that need to form hydrogen bonds (Table 2) The high proportion of non-polar side chains that occur in protein interiors essentially results from the necessity of forming a close packed structure in which nearly all buried polar groups form hydrogen bonds

It follows that structural homology between proteins of different amino acid sequence is essentially a packing phenomenon

Protein folding

The folding problem of a globular protein is how to gain enough energy from hydrophobicity, hydrogen bonds and van der Waal's interactions to overcome the loss of configurational entropy and the steric strain that occurs in the folded state The calculations described here show that the structural solution to at least part of this problem is described by a set of simple expressions, and raise the question are there regularities present in the topology of the folded chain—in the amount of secondary structure and

the manner in which it is assembled—which are responsible for these simple expressions?

Much of this paper arose from discussions with Michael Levitt, Frederic M Richards and Joel Janin and the gifts of computer programs by the first two I thank Professors D C Hodgkin, K Hardman, M G Rossmann and B W Matthews for allowing me to use unpublished atomic coordinates Most of this work was supported by an EMBO fellowship and part by a grant to F M Richards from the National Institutes of Health

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Inhibition of release of prostaglandins as an explanation of some of the actions of anti-inflammatory corticosteroids

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Corticosteroids as well as non-steroid anti-inflammatory drugs inhibit the prostaglandin-mediated vasodilatation accompanying lipolysis in subcutaneous fat Whereas the non-steroids produce their effect by inhibition of prostaglandin synthesis, however, corticosteroids inhibit their release This mechanism may be the basis of some actions of corticosteroids in inflammation, in the gastric mucosa and in the CNS

A PROSTAGLANDIN (PG), probably E_2 (refs 1 and 2) is a mediator of functional vasodilatation (vasodilatation accompanying activity) in subcutaneous adipose tissue In 1971 it was discovered[3] that non-steroid anti-inflammatory agents such as aspirin and indomethacin prevented PG formation by inhibition of PG synthetase It was subsequently shown that such anti-inflammatory agents inhibit PG formation and functional vasodilatation in subcutaneous fat but do not prevent lipolysis[4] Therefore, it seems likely that these actions are the result of inhibition of PG synthetase activity in adipose tissue We have now shown that functional vasodilatation in fat is inhibited not only by non-steroid anti-inflammatory drugs of the aspirin type,

but by the anti-inflammatory steroids hydrocortisone, betamethasone and prednisolone as well

Inhibition of vasodilatation

The method used was that described earlier[1] in which the venous outflow from the epigastric adipose depot of rabbits was measured directly with a drop counter and fat mobilisation was induced by a close-arterial infusion of adrenocorticotrophic hormone ($ACTH_{1-24}$) This lipolysis was accompanied by PGE_2 formation in the fat tissue and dilatation of the blood vessels giving an increased blood flow

When hydrocortisone (2 nmol min^{-1} to 2 μmol min^{-1}) as hemisuccinate was infused into the fat depot immediately before the infusion of $ACTH_{1-24}$, the vasodilatation was greatly reduced or abolished The blood steroid level during the infusion was about the same as that in a rheumatoid patient on steroid therapy Figure 1 shows the percentage inhibition of the vasodilatation produced after activating the fat tissue with a close-arterial infusion of $ACTH_{1-24}$ (1 μg min^{-1}), brought about by 15 min infusions of various concentrations of hydrocortisone

The first interpretation of this finding was that anti-inflammatory steroids as well as aspirin-like drugs inhibit PG synthetase in adipose tissue It has been suggested that PG biosynthesis in
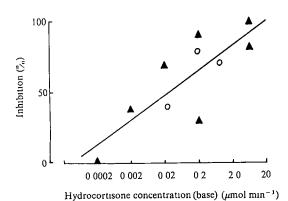
Fig 1 The effect of hydrocortisone (as hemisuccinate) on the vasodilatation in subcutaneous adipose tissue (▲), and on the output of a prostaglandin-like substance into the venous effluent blood (○), during lipolysis brought about by a 5 min close-arterial infusion of $ACTH_{1-24}$ Each point represents the result from one rabbit The inhibition is plotted as a percentage of control and the concentration of hydrocortisone in μmol min⁻¹ is plotted on a log scale The line is the regression line calculated from the points plotting the inhibition of vasodilatation

human skin is inhibited by corticosteroids[5] The same research group[6], however, working with a microsomal fraction from skin, have more recently agreed with other groups who found that anti-inflammatory steroids do not inhibit the PG synthetase activity of homogenates prepared from lungs, spleen or other tissues When we studied prostaglandin formation and leakage from another tissue, the rabbit jejunum, as described earlier[8], our results were in agreement with theirs We found that although indomethacin (30 nmol ml⁻¹) inhibited the leakage of PG from segments of rabbit jejunum suspended in 15 ml Krebs solution at 37 °C, hydrocortisone caused no reduction This finding implied that unlike indomethacin, hydrocortisone did not inhibit PG synthetase

We therefore analysed further the hydrocortisone-induced inhibition of the PG-mediated functional vasodilatation 'n adipose tissue We wanted to determine whether hydrocortisone was inhibiting the vasodilatation by inhibiting lipolysis itself, or by antagonising the action of PG, or by preventing its formation, or by interfering in some way with the release of PG after its formation
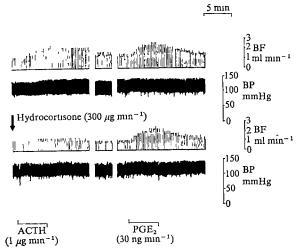
Fig 2 Record of venous outflow (BF) upper record, from the right epigastric fat pad and arterial blood pressure (BP) in a 4 5 kg female New Zealand White rabbit Responses to 5 min close-arterial infusions of $ACTH_{1-24}$ (1 μg min⁻¹) and PGE_2 (30 ng min⁻¹) Upper tracings before and lower tracings after close-arterial infusion of hydrocortisone hemisuccinate (300 μg min⁻¹) (equivalent to hydrocortisone 600 nmol min⁻¹) The gaps between each block of tracing is equivalent to 30 min The increased venous outflow following $ACTH_{1-24}$ is almost abolished whereas the response to PGE_2 is unaffected

First, we were able to show that hydrocortisone does not inhibit lipolysis When hydrocortisone (600 nmol min⁻¹) was infused close-arterially to the epigastric fat depot immediately before the lipolytic substance ACTH (1 μg min⁻¹), the free fatty acid content of the epigastric venous blood reached a level of 3–5 meq l⁻¹, that is the same level reached after stimulating with $ACTH_{1-24}$ in the absence of hydrocortisone

Second, the inhibition of vasodilatation was not the result of a direct antagonism of the action of PG by the corticosteroids In three experiments, infusions of hydrocortisone (600 nmol min⁻¹) (the ED_{75} used to inhibit functional vasodilatation) did not inhibit the vasodilatation produced by close-arterial infusions of PGE_2 at 30–100 ng min⁻¹ A typical experiment is illustrated in Fig 2 In a second series of experiments betamethasone (as disodium phosphate) given at a rate of 2–20 nmol min⁻¹ inhibited the vasodilatation accompanying lipolysis more than 90 % whereas the vasodilatation produced by infusions of PGE_2 (30–100 ng min⁻¹) was not reduced at all In some experiments it was even potentiated

Third, using the method of Bowery and Lewis[4] it was possible to show that hydrocortisone did not inhibit the formation of PG in the fat tissue during lipolysis The epigastric fat depots of both sides of the animal were isolated from the skin and abdominal wall and prepared for close-arterial injection of a fat-mobilising agent $ACTH_{1-24}$ (1 μg min⁻¹) was infused close-arterially to the right epigastric fat pad for 5 min, and 15 min after beginning the infusion the fat pad was excised and placed immediately into ice-cold ethanol, chopped finely and homogenised at 0 °C using an Ultra Turrax homogeniser After allowing the rabbit to recover for 1 5 h after the $ACTH_{1-24}$ infusion,

Table 1 Prostaglandin activity in fat pad extracts in equivalents of PGE_2 ng per g tissue formed after activation with $ACTH_{1-24}$

Experiment	$ACTH_{1-24}$ (1 μg min⁻¹)	Hydrocortisone (6 0 μmol min⁻¹) + $ACTH_{1-24}$	Indomethacin (30 nmol min⁻¹) + $ACTH_{1-24}$
1	38 1	45 5	
2	36 0	47 0	
3	8 0	7 1	
4	47 6		< 0 25

hydrocortisone (600 nmol min⁻¹) was infused close-arterially to the left fat pad for 15 min followed immediately by $ACTH_{1-24}$ (1 μg min⁻¹) for 5 min Again 15 min after the beginning of the infusion of $ACTH_{1-24}$ the fat pad was excised and placed immediately into ice-cold ethanol, chopped and homogenised as before Both fat pads were examined for prostaglandin content by extraction into ice cold ethanol and ethyl acetate at pH 3 The extracts were evaporated to dryness under reduced pressure at a temperature not exceeding 45 °C The residues were taken up in 0 1 ml ethanol and applied to thin-layer chromatographic plates using the A1 solvent system[9] PGE_2 and $PGF_{2\alpha}$ standards were chromatographed simultaneously with the extracts and were made visible by exposure to iodine vapour The silica gel was scraped from the thin-layer plates at 1–2 cm intervals and suspended in 1 ml Krebs solution The PG activity of the supernatant was estimated by bioassay on the isolated rat stomach strip (RSS), rat colon (RC) and chick rectum (CR) All the PG activity from the fat pad extract appeared in fractions corresponding to the PGE_2 standard

The results given in Table 1 show that hydrocortisone does not prevent the formation of PG when the fat tissue is activated The increased PG content of extracts of fat pads which occurs on activation[2] was not inhibited by hydrocortisone On the other hand, as shown in the Table, a similar experiment confirms the earlier observation[4] that indomethacin prevents or reduces the formation of PG

Prostaglandin release

Since the corticosteroids inhibit the vasodilatation but not the formation of PG in the tissue, it seems possible that they prevent the release of PG from the fat cells thereby preventing their
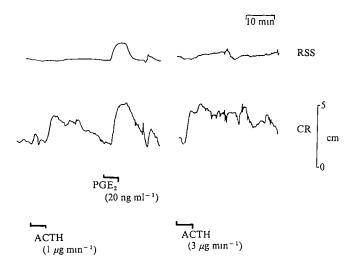
Fig 3 The venous effluent from the epigastric fat pad of an anaesthetised New Zealand White rabbit (female, 4 3 kg) was superfused over two isolated assay tissues, rat stomach strip (RSS) and chick rectum (CR) and returned to the animal by way of a jugular vein When PGE_2(20 ng ml^{-1})was infused into the blood bathing the assay tissues (IBB) both RSS and CR contracted When $ACTH_{1-24}$ (1 and 3 μg min^{-1}) was infused close-arterially to the fat pad for 5 min a substance causing contraction of CR (approximately equivalent to PGE_2 20 ng ml^{-1}) but not RSS was released into the venous blood In other experiments $ACTH_{1-24}$ did not contract the assay tissues when given IBB Vertical scale 5 cm, time 10 min

action on the blood vessels A suggestion that this might be true arises from a study of the release of a PG -like material from the fat pad into the blood during lipolysis The method was to prepare the fat depot as before for recording venous outflow and for making close-arterial infusions In this series of experiments, however, the venous blood from the fat depot was superfused over a series of isolated smooth muscle tissues, RSS, CR and RC[10] before being returned to the animal The tissues were made more specific for PG-like materials by bathing them before superfusion in a solution containing antagonists of histamine, 5-hydroxytryptamine (5-HT), acetylcholine and catecholamines and infusing these inhibitors through the lumen of CR and RC during the experiment[11] Figure 3 illustrates the release into the venous effluent of a substance which contracts the isolated chick rectum which is sensitive to PGs of the 'E' type Like the vasodilatation[4], the output of this material was reproducible when repeated stimulations by ACTH were made at hourly intervals When the venous effluent was tested on the RSS, RC and CR and compared with responses to PGE_2 it was obvious that the substance in the venous blood was not PGE_2, although the substance formed in the fat tissue itself is PGE_2 (ref 2) as recently confirmed[12] using mass spectrometric analysis Although the venous effluent blood consistently contracted CR and to a lesser extent RC, it caused little or no contraction of RSS In addition, when the epigastric venous blood collected during lipolysis was extracted with solvent systems similar to those used for extraction of PGs[13], a little PG-like activity was retained The substance however, was not PGE_2, compared with which it was more active on CR and RC than on RSS Neither $ACTH_{1-24}$ itself nor any substance released during infusion of $ACTH_{1-24}$ altered the responsiveness of the isolated tissues to PG

In further experiments of this type we have found that the output of this material during activation of the adipose tissue is prevented by previous infusion of indomethacin This finding strongly suggests that although the material is not PGE_2, it is a product of the PG synthetase system This effect was observed not only with indomethacin, however, since infusions of hydrocortisone or betamethasone before the $ACTH_{1-24}$-induced activation of the adipose tissue also prevented the output of the PG-like material as illustrated in Fig 4 Figure 1 shows that

when the output is plotted against concentration of hydrocortisone, the points fall along the same curve obtained from a plot of inhibition of vasodilatation

In spite of its resemblances to PGs, the possibility exists that the chick rectum contracting substance is an unrelated new kind of chemical mediator the release of which is inhibited by both steroid and non-steroid anti-inflammatory agents

Active transport inhibition

In summary we have found that like non-steroid anti-inflammatory drugs such as aspirin and indomethacin, anti-inflammatory steroids such as hydrocortisone, betamethasone and prednisolone inhibit the PG-mediated functional vasodilatation in subcutaneous adipose tissue The mechanism of action of the steroids is, however, different Like indomethacin, hydrocortisone inhibits vasodilatation, prevents the output into the blood of a PG-like substance, possibly a metabolite, and does not antagonise the vasodilator action of PG directly But whereas indomethacin inhibits the formation of prostaglandin in the fat tissue itself, hydrocortisone does not, it therefore seems likely that hydrocortisone inhibits the release of prostaglandin Perhaps it does this by preventing the transport of prostaglandin from inside the fat cell to the extracellular space where it would normally act on the blood vessels This is the first indication that formation and release of PGs may be distinguishable since previously the two processes were thought to be equitable[13]

Since the discovery that non-steroid anti-inflammatory drugs produce at least some of their anti-inflammatory effects by inhibition of PG synthetase thereby preventing the formation of PGs, it has been difficult to explain the same kind of anti-inflammatory activities of corticosteroids, since it has been demonstrated by several groups of workers that anti-inflammatory steroids do not inhibit PG synthetase

In situations where PG formation occurs extracellularly or where the cells are damaged so much that their membranes no longer provide a barrier to the diffusion of materials, PG synthetase inhibitors would prevent the appearance of PGs For example, in our experiments with strips of rabbit jejunum, it seems likely that PGs are probably formed during cell breakdown and their leakage from these damaged cells could not be expected to be inhibited by steroids, although their formation and therefore their leakage is inhibited by indomethacin It has also been shown that steroids did not affect PG levels in an inflammatory exudate *in vivo*[14], that is, another situation where there is cell necrosis But Ferreira *et al*[15] found a similar result in perfused spleen where cell damage would seem unlikely,
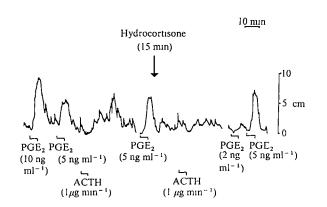
Fig 4 The venous effluent from the fat pad from a female New Zealand White rabbit (3 4 kg) was superfused over a single CR The tracing is arranged and labelled as in Fig 3 In the first panel calibrating doses of PGE_2 of 5–10 ng ml^{-1} were given IBB When $ACTH_{1-24}$(1 μg min^{-1} for 5 min) was infused close-arterially to the fat pad a substance which caused a contraction of CR equivalent to 5–10 ng ml^{-1} PGE_2 was released into the venous blood In the second panel, hydrocortisone (500 nmol) was infused close-arterially to the fat pad for 15 min immediately before $ACTH_{1-24}$ (1 μg min^{-1}) This time there was no release of contractile substance 1 5 h elapsed between doses of $ACTH_{1-24}$

although the perfusions with Krebs solution and dextran might have caused some alterations in the cells On the other hand, during lipolysis there is no damage to the fat cells and the release of PGs from these cells is therefore probably an active process rather than leakage It is this 'active transport' which seems to be inhibited by corticosteroids It seems likely that this effect on subcutaneous adipose tissue might well account for some anti-inflammatory effects of corticosteroids in skin, in particular their local vasoconstrictor activity[16] The inhibition of PG release may well have further implications on the mode of action of corticosteroids on inflammatory cells and the cells of other systems like those of the reproductive tract

Such a hypothesis needs confirmation by experiments with isolated cells First it would be required to show that isolated fat cells synthesise PG when stimulated and that its release is inhibited by anti-inflammatory steroids It would also be interesting to examine the effect of steroids on the release of PGs from cells which play an obvious role in inflammatory reactions, such as mast cells, leukocytes and platelets

Until now there has been no report of a transport mechanism for the release of PG from cells, formation being synonymous with release But some investigations have indicated that there might be an active transport mechanism for the uptake of PGs[17,18] After injection of ^{3}H-labelled PG radioactivity was found to be concentrated in some tissues In addition, PGs were found to be rapidly taken up into lungs but the parent compounds and metabolites were released slowly On the other hand, there have been many reports of the membrane stabilising effect of anti-inflammatory steroids like hydrocortisone[19] It is not clear precisely what is meant by 'stabilisation' but it might well be involved in the inhibition of PG release

Inhibition of PG synthetase by non-steroid anti-inflammatory drugs may not only be the basis of their anti-inflammatory activity but may also be the cause of their main side effect–gastric-irritation If PGs mediate functional vasodilatation in the gastric mucosa as has been suggested[20], PG synthetase inhibitors would probably produce areas of ischaemia leading to necrosis As anti-inflammatory steroids also produce gastric ulceration, it

is possible that they act in the gastric mucosa too, by inhibiting the release of PG

Another instance where the inhibition of PG release by anti-inflammatory steroids might explain their action is in their effect in controlling fever It has been suggested that pyrogenic fever is caused by release of PGs in the anterior hypothalamus[21,22] In addition, it has been shown that PG synthetase inhibitors reduce or prevent the release of PGs into the cerebral ventricles and reduce fever[23,24] The release of PGs in the hypothalamus may well be mediated through a similar sort of transport mechanism as that which seems to be present in adipose tissue It would therefore be interesting to examine the effect of corticosteroids on PG release in the brain during fever since it is known that certain kinds of fever are alleviated by these agents

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letters to nature

Silicon-rich stellar envelope?

DURING an analysis of Orion-2 data derived from a space astrophysical experiment[1], we came across a hot star with a gaseous envelope, SAO077308, of B1e type and nearly ninth magnitude It is interesting because of an extremely strong emission line at 2,520 Å occurs in its ultraviolet spectrum Following a series of attempts, the identification of this line with the resonance sextet of neutral silicon (mean wavelength 2,520 Å) seems probable The components of this sextet, of almost equal strengths, are 2,507, 2,514, 2,516, 2,519, 2,524 and 2,528 Å, the second of these lines is resonant, the others quasi-resonant in the sense that the lowest levels of these lines are located quite close to the ground level, up to 0 01–0 03 eV This identification cannot be taken as final and needs further examination Irrespective of possible closer definitions, however, the very presence of the emission line itself in the spectrum of this star is significant Regardless of the true nature of the chemical element to which this line belongs, the problem of an anomalous abundance of this element cannot be overlooked

Here we report the further somewhat conventional examination of the abnormal abundance of silicon in the envelope of the star SAO077308

The emission line at 2,520 Å is the strongest in the spectrum of SAO077308 at least in the range from 4,000 Å to 2,300 Å, one can see its unusual strength in the densitometric recording of the spectrum of this star in Fig 1 (the spectral resolution is nearly 15 Å at 2,500 Å) To establish the anomalous nature of the line in the spectrum of this star, a comparison with the spectrum of another well known emission star, ζ Tau, of almost the same spectral class, has been made This is shown in Fig 2 by collating the densitometric recordings of the same parts of both spectra near 2,500 Å

A few spectral images with an exposure time of 7–8 min (frame F23) have been obtained for the star SAO077308 The spectral pictures of ζ Tau were obtained from Orion-2 with an exposure time of nearly 5 s (F4), that is, in conditions considerably more favourable than in the case of SAO077308, bearing in mind the inevitable deterioration of the quality of the spectrograms during long exposures as a result of the
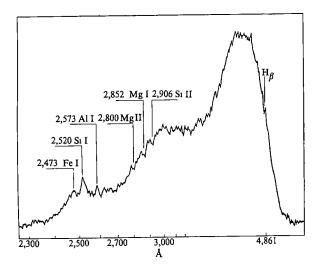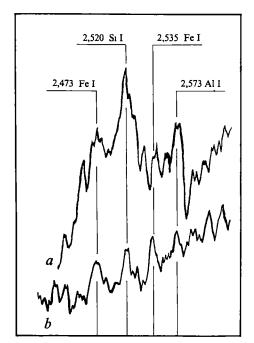
Fig 1 Densitometric recording fiom the ultraviolet spectra of emission star SAO077308 in the range of wavelengths from 5,000 Å to 2,300 Å The strongest emission line 2,520 Si I is visible

accidental disturbances of the guide system of Orion-2 Nevertheless, the line at 2,520 Å is sharply distinguished compared with nearby emission lines in SAO077308, but this line has the same intensity as those of nearby emission lines in the spectrum of ζ Tau The alternative assumption that this difference may be the result of the anomalously small abundance of iron (2,473 Fe I, a resonance doublet) and aluminium (2,573 Al I, a resonance triplet) in SAO077308 seems unlikely

It is difficult to determine the relative abundance of silicon in the envelope of the star SAO077308 as compared to ζ Tau, in view of the different conditions in which the spectral images were taken A very approximate estimate shows that the relative abundance of silicon in the envelope of SAO077308 must be at least 3–4 times, and perhaps a whole order of magnitude, larger than in the envelope of ζ Tau

To what degree the discovered abnormal abundance of

Fig 2 Comparison of two densitometric recordings from the spectras of the stars SAO077308 (a) and ζ Tau (b), near 2,500 Å, from which the anomalous intensity of 2,520 Si I in the case of SAO077308 is clear

silicon is characteristic, not only of the gaseous envelope of the star SAO077308 but also of the photosphere itself, is difficult to specify without additional data To achieve this, it is necessary to look for the absorption lines in the spectrum of the star under examination by means of ground based astrophysical observations and to compare those lines with their analogues in the spectrum of the comparsion star

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Ionospheric mid-latitude trough and the abrupt scintillation boundary

THE mid-latitude trough[1,2] in electron density and the equatorward limit of small-scale F-region irregularity structure apparent in the scintillation of satellite and radio star signals, the scintillation boundary[3,4], are both features of the transition region between the high and mid-latitude ionospheres There are broad similarities between the morphology and statistical behaviour of the trough and the irregularity boundary[5-7] and, although the individual behaviour of the scintillation boundary and the mid-latitude trough may be related to particle precipitation patterns or plasmapause movements, there is a definite local time variation in their mean relative latitudinal positions when simultaneous observations of the two phenomena are analysed[8] We have examined this variation in more detail, using data from simultaneous observations of trough and scintillation boundary positions

Some 530 recordings of passes of the ionospheric beacon satellite BE-B (1964-64A) taken at Aberystwyth (52 42°N, 4 05°W) were selected from the available records for the years 1966 to 1968 inclusive The sample analysed comprised those records which showed irregular Faraday fading rates, indicating steep gradients in total electron content, or a sharp transition from zero to full scintillation of the 40 MHz signal indicating the presence of a clear scintillation boundary Reduction of the 40 and 41 MHz records by means of the differential Faraday technique yielded the variation of total electron content with invariant latitude, for an assumed ionospheric height of 350 km, over the approximate range 45° to 63° These plots of total electron content were used to determine the trough latitude, for the purposes of the analysis this was taken to be that corresponding to the minimum electron content recorded in the trough The invariant latitude, at 350 km, of the scintillation boundary was also determined The definition of the boundary used, a mean between zero and full modulation of the signal, was of the 'abrupt' type[9], with 95% of the sample having widths less than 3°, the width being the latitudinal range over which the scintillation depth varied from zero to full modulation of the 40 MHz signal

The results (Fig 1) show the contrast between the diurnal behaviour of the trough and scintillation boundary latitudes as a function of time The trough was first observed at ~59°Λ at 1900 LMT, falling rapidly in latitude to ~55°Λ at 2300 LMT and then more slowly to reach a minimum ~54°Λ at 0600 LMT, subsequently it returned very rapidly to higher latitudes A separate study of the gradients of the trough walls indicates that this apparent movement to higher latitudes is a filling of the equatorward margin of a stationary trough formation by solar electromagnetic radiation produced ionisation as dawn advances, rather than a true poleward motion of the trough as a whole In contrast to trough behaviour, the scintillation boundary has been observed at all times of the day, it
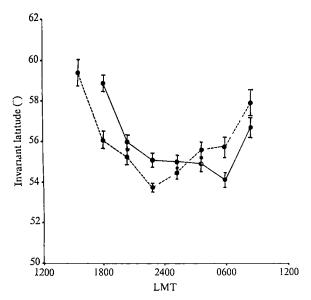
Fig 1 Variations of the latitude of the mid-latitude trough and the scintillation boundary as functions of local mean time The solid line represents the trough, the dashed line the scintillation boundary The points plotted represent median values for each time interval, using only data for which the planetary magnetic index, K_p, was less than four The bars denote the standard errors of the median values

is found at 59 5°Λ at 1600 LMT, falling steadily to a minimum at 53 5°Λ by 2300 LMT and retreating more slowly to reach ~58°Λ by 0900 LMT These results are in broad agreement with the mean latitudinal differences in trough and scintillation boundary positions reported previously[8] It now seems that the differences in the relative behaviour noted earlier, based on simultaneous observations, also occur in the absolute statistical variation of these phenomena, furthermore the clear differences in the diurnal behaviour of the trough and the scintillation boundary can explain these results

We conclude that the mid-latitude trough and the scintillation boundary, although both features of the high to mid-latitude ionosphere interface, are essentially independent having distinct diurnal behaviour patterns It must therefore be supposed that the immediate causative mechanisms, whether in terms of particle precipitation, plasmapause behaviour, electric field effects or some more complex process involving plasma waves, are also essentially unrelated

The movement of the scintillation boundary, reaching minimum latitude around local midnight, is similar, although displaced towards the equator, to that of the edge of the auroral oval closest to the equator It is possible that an energy source at E-layer altitude, within the auroral oval, acts by means of electric fields or perhaps plasma waves to cause the observed irregularities which lead to scintillation It does not seem that the trough can be related directly to reported particle precipitation patterns, but its movement shows certain similarities to that of the plasmapause, at least in the midnight to post-dawn sector But the present results do not reveal any motion of the trough which can be related to the 'dusk bulge' in plasmapause position We therefore conclude that one-to-one correspondence between trough and plasmapause movements may not be maintained at all times

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Interaction of a photon with a gravitational field

IT is usual to analyse the behaviour of photons in gravitational fields as if they were point particles which travel along geodesics But by taking into account the finite spatial extent of the photon I show that it could interact with the transverse gradient of the gravitational field to give a deflection additional to the well known gravitational deflection of General Relativity I postulate that this deflection, even though it is extremely small, could be the basis for a much more important effect in which the photon can decay into three photons (or possibly (E H Derrick, personal communication) several photons with one or more gravitons) If such a mechanism is possible it would appear as a decrease in photon energy and could be of astrophysical importance in explaining many of the anomalous redshifts that have been observed[1,2]

In some situations the secondary photons which are expected to have very low energies may be observed as low frequency radio waves

For a classical electromagnetic wave its linear momentum **p** and its angular momentum **L** are given by the two volume integrals

$$\mathbf{p} = \int \varepsilon\mu\mathbf{N}d\tau \qquad (1)$$

$$\mathbf{L} = \int \varepsilon\mu\mathbf{r}\times\mathbf{N}d\tau \qquad (2)$$

where **N** is the localised Poynting vector ($\mathbf{N}=\mathbf{E}\times\mathbf{H}$)

Moller[3] has shown that in General Relativity for the description of electromagnetic radiation a weak stationary gravitational field may be considered as a refractive medium with $\varepsilon\mu=1/c^2/(1-2\chi/c^2)$ where χ is the gravitational potential Clearly this description would be valid for many other theories of gravitation which predict the deflection of light by a massive body Now in a region of space which is large compared to the possible extent of a photon but small compared to variations in the gravitational field, the gravitational potential can be expressed as $\chi=\chi_0+\nabla\chi$ **r**, where **r** is vector centred in the region

$$\mathbf{p}=\left[1-(2\chi_0/c^2)\right]\mathbf{p}_0-(2/c^2)\int(\nabla\chi \ \mathbf{r})\mathbf{N}d\tau \qquad (3)$$

where $\mathbf{p}_0$ is the momentum in a region containing no field and the first term on the right is the usual gravitational redshift, which is of no further concern here Using the vector triple product expansion the second term can be expanded to give

$$\int(\nabla\chi \ \mathbf{r}) \ \mathbf{N}d\tau = \int(\nabla\chi \ \mathbf{N}) \ \mathbf{r} \ d\tau - \int\nabla\chi\times(\mathbf{r}\times\mathbf{N})d\tau \qquad (4)$$

applying these equations to a circularly polarised photon which has circular symmetry about its trajectory the first term on the

right of equation (4) is zero and equation (2) can be substituted in the second term to give

$$p=[1-(2\chi/c^2)]p_0-(2/c^2)\ \nabla\chi\times L \qquad (5)$$

Since a photon has angular momentum parallel to its trajectory equation (5) predicts an additional deflection orthogonal to both the photon trajectory and to the gradient of the gravitational field (the vector taking opposite sign for right handed and left handed polarisation) Thus for a photon that just grazes the edge of the Sun there is no change between the initial and final directions but there is a small lateral displacement For a photon of wavelength λ this is $2GM\lambda/\pi R$ where G is the gravitational constant, M is the mass of the Sun and R is its radius The separation between right handed and left handed polarisations of a source at the Sun is twice this value and corresponds to an angular separation of $1\ 5\times10^{-11}$ arc s for $\lambda=37$ mm This is very much smaller than a recent[4] upper limit of 2×10^{-3} arc s

The angular momentum may be calculated by substitution in equation (2) but in this case the term proportional to the gradient of χ is zero because of the circular symmetry about the trajectory of the poynting vector N. This leads to the 'paradox' that the photon spin is no longer parallel to the photon trajectory as defined in equation (5) One could imagine the reference trajectory to be defined by the photon spin and the last term in equation (5) to be the result of a perturbation caused by an interaction with the gravitational field Havas[5] has shown that if there is a momentum transfer, as occurs here, there is no known reason why the photon cannot split up into three or more photons It is the conservation of spin, momentum and energy which gives the photon the characteristics of a single particle I therefore postulate that a photon can interact with a gravitational field to produce two or more secondary photons It seems reasonable to assume that the rate of energy loss per unit path length resulting from the production of secondary photons is proportional to the magnitude of the transverse momentum given by equation (5), and to make the proportionality constant dimensionless the path length is measured in wavelength units Alternatively, one could argue that the distance between interactions is that which would give a lateral displacement of the order of a wavelength and each interaction produces on average an energy loss proportional to the energy of the primary photons Both arguments lead to the same equation for the energy loss, namely

$$(1/E)\partial E/\partial l = - (K/c^2)|\nabla\chi\times n| \qquad (6)$$

where l is the distance measured along the photon trajectory, K is a dimensionless constant and n is a unit vector parallel to the photon trajectory This decay process can be likened to the removal of a tidal strain set up in the photon by the gradient of the gravitational field and it must therefore compete with any other process that could remove the strain In particular if there is a refractive medium present the excess momentum could be transferred to the medium without the decay process occurring In order to estimate the constant K this postulate can be used to explain the anomalous redshift[1] observed in solar emission lines coming from the limb of the Sun Then by integrating equation (6) the redshift may be expressed as an effective velocity v where

$$v=(KGM/cR)\ ((1-\cos\theta)/\sin\theta) \qquad (7)$$

where θ is the angle between the line of sight and the solar radius at the point of observation

Since the experimental observations are subject to an unknown additional constant the 25 velocities given by Adam[1] in his Fig 6 were fitted by the least squares method to give

$(0\ 962\pm0\ 083)[(1-\cos\theta)/\sin\theta]+(0\ 416\pm0\ 065)$ km s^{-1}

Then equating the first coefficient to the constant in equation (7) gives $K=1\ 51\pm0\ 13$ Although the r m s residual (0 05 km s^{-1}) is small, the experimental scatter is too large to confirm the functional form of equation (7)

Since the secondary photons will have frequencies lower than the plasma frequency of the solar corona there will be virtual emission with all the energy loss being used to heat the corona Integration of equation (7) shows that the energy loss to the solar corona measured per unit area of the photosphere is $(KGM/cR)/(\pi/2-1)\ F$, where F is total radiation flux density of the primary photons For the Sun with $F=6\ 41\times10^7$ W m^{-2} the loss to the corona is 117 W m^{-2} (with $K=1\ 5$) or a total power of $7\ 08\times10^{20}$ W At the solar minimum the estimated loss[7] to radiation and the solar wind is 200 W m^{-2}, however, allowance must be made for conduction losses to the chromosphere, values for which vary from[6] 50 W m^{-2} to an order of magnitude greater[7] Athay[8] has argued that this conduction loss may not be a genuine loss but that the energy is returned to the corona by mass motions of the chromospheric filaments In any case these low energy photons could be an important energy source for the solar corona

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Is the Earth tide phase lag unaffected by anelasticity?

THE dynamics of the Earth–Moon system raises the question whether the bodily tides or ocean tides in the Earth–Moon system produce most of the energy dissipation which is necessary to account for the secular retardation of the rate of rotation of the Earth Macdonald[1] has shown that a phase lag of 2° of bodily tides is enough to account for the whole change, and in principle this phase lag could be obtained from earth tide observations In practice, however, the determination of the phase lag of Earth tides is extremely uncertain, since the true value of the delay is influenced by the lag of recording instruments and by several disturbing effects Parussky[2] found that the mean value of the phase lag of solid Earth tides in Europe and in Asia is approximately equal to that of mentioned above, but the individual values are so much different one from another that the averaging is rather uncertain The study reported here indicates that all of the phase lag derived from Earth tide observations may be instrumental in origin

Miller[3] investigated in detail the possibility of dissipation in shallow seas His results hint that shallow seas are responsible for two-thirds of the dissipated energy, this numerical value, however, is so uncertain that as little as one-third or as much as

100% of the dissipated energy may be attributed to the shallow seas

I have obtained the Earth tide phase lag in a different way, computing the phase delay in case of a supposed model of the internal structure of the Earth and of its rheological characteristics The phase lag of crustal deformations has been computed using Molodensky's[4] theory As a starting point I used the homogeneous equations for the deformations of an elastic Earth of spherical symmetry[4] leading to a set of six linear differential equations In this equation system six unknown functions occur, these being proportional to the radial and tangential components of the displacement vector, to stress, to the tide generating potential and to its derivatives, respectively For example, one of the functions sought for and denoted by H satisfies the following connection with the U_r radial component of the displacement vector

$$U_r = H_n(a^{n-1}/r^n)/(W_n/g)$$

where a is the radius of the Earth, r is the distance from the centre of the Earth to the point of interest, g is acceleration due to gravity, n is the degree of harmonic development of the tidal potential W Introducing complex expressions of the shear modulus and of the unknown functions we have

$$\bar{\mu} = \mu_o(1+im) = \bar{\Phi}_j = \Phi_j + i\Phi_j^* = j = 1,2, \quad ,6, \quad (1)$$

and we obtain for the imaginary parts of the corresponding functions an inhomogeneous differential equation system taking into account the effect of anelasticity too In equation (1) μ_o represents the real part of the shear modulus, $i = \sqrt{-1}$, m is some chosen function and Φ_j denote the functions sought for The bulk modulus is taken here as being a real number This means that energy dissipation in compression is much less than in shear Solving the homogeneous differential equations relating to the real parts of the functions being sought we can compute the values of the real parts of Love numbers The imaginary parts can be obtained using the method of variation of constants, after having obtained the solution of the problem relating to the deformations of ideally elastic Earth

Full details of the method are published elsewhere[4,5], here I restrict myself to the description of results of the computations

I assumed the Earth model of Bullen and Haddon[6] denoted as model B_2 I also assumed that rheological features of the Earth can be characterised by the Knopoff–Lomnitz body, that is, the Q dissipation function does not depend on the frequency of oscillations Furthermore, it is known that in case of a Knopoff–Lomnitz body function m figuring in equation (1) is connected with Q by

$$m = Q^{-1}$$

The radial variation[7] of the function Q is given in Table 1 Solving the equations of the deformations of the elastic Earth gives the values of the Love numbers the values included in Table 2 These values are in good agreement with those obtained from observations On the basis of the processing of a 50,000-d long series of Earth tide measurements, Melchior[8] gives the following values for k and h

$$k = 0\ 316 \pm 0\ 010,$$
$$h = 0\ 637 \pm 0\ 016$$

After solution of the problem relating to the ideally elastic Earth, I turned to the solution of that dealing with deformations

Table 1 Radial variation of dissipation function Q

Depth (km)	Q
0–38	450
38–300	100
300–1,000	300
1,000–2,898	1,000

Table 2 Computed values of the second and third order Love numbers

n	h	l	k
2	0 6335	0 0882	0 3228
3	0 2983	0 0150	0 0970

of an inelastic globe, using the Q profile given in Table 1 The imaginary parts of the Love numbers obtained are almost zero Consequently, the phase lag of tidal deformation—which can be given, as a matter of fact, as the ratio of the imaginary and real parts of Love numbers—vanishes, and for practical purposes we have no phase lag For example, the computed value of the phase lag of gravimetrically obtainable δ factor is only 1–2 These results suggest that at present the solid Earth tides do not play a significant role in the deceleration of the Earth

It is difficult to say to what extent the computed phase lag values might be in accordance with observational results, since the determination of instrumental lag is rather uncertain The instrumental lag of recording apparatuses can be computed mathematically or it can be determined experimentally using some artificial gravity oscillation But in both cases an insufficient knowledge of parameters and physical characteristics of the instrument makes the exact determination of the accurate instrumental lag rather doubtful

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Age differences between Archaean cratons of eastern and southern Africa

ARCHAEAN greenstone belts have commonly been correlated on the basis of similarities in their stratigraphic successions[1] Conclusive radiometric tests of these correlations have rarely been made, because of the difficulty of dating the rocks of the greenstone belts themselves, and because of uncertainties in their relationships to the more easily dated granitoid rocks with which they are normally associated We report here radiometric evidence from eastern Africa, which clearly contradicts previous lithostratigraphic correlations between the Tanzanian and Kaapvaal Cratons by establishing a minimum age difference between the uppermost parts of their respective greenstone belt successions

In Table 1 the stratigraphy of the greenstone belts of the Tanzanian Craton (see Fig 1) is compared with those of the Rhodesian and Kaapvaal cratons The Kavirondian and Nyanzian systems, confined to the northern part of the Tanzanian Craton, are particularly well exposed near the Tanzanian–Kenya border towards Lake Victoria There is no direct evidence that the Dodoman is older than the Nyanzian[2], only the presence of metamorphosed ultrabasic rocks in its type area[3] supports the analogy with the Sebakwian and Lower Onverwacht suggested in Table 1

The Kavirondian and Nyanzian systems were shown by Cahen and Snelling[4] to be older than about 2,550 Myr Subsequently, Old and Rex[5] published a 3,100 Myr Rb–Sr isochron age for the Masaba granite, south-eastern Uganda, which they considered a minimum age for the Nyanzian Field relationships suggest that the granite may be post-Kavirondian[6], which means that the greenstone belts in eastern Africa would be comparable

Table 1 Comparison of some African greenstone belts*

Tanzanian Craton	Rhodesian Craton	Kaapvaal Craton	Lithology	
Kavirondian System (Unconformity)	Shamvaian System (Unconformity)	Moodies Group (Unconformity)	Sedimentary Group coarse grits, conglomerates and shales	
		Fig Tree Group	Turbidites	
Nyanzian System	Bulawayan System	Upper Onverwacht Group	Greenstone Group mafic and salic flows and tuffs, banded ironstones and cherts	
Dodoman System	Sebakwian System	Lower Onverwacht Group	Ultramafic Group dunites, peridotites, tholeitic basalts, minor sediments	

*Modified after Anhaeusser *et al* [1]

in age to the very ancient rocks of the Swaziland System[7-10] (All Rb–Sr ages are calculated here with $\lambda_{Rb} = 1\,39 \times 10^{-11}\,yr^{-1}$, to facilitate comparison with published data on southern Africa)

We have dated pre and post-Kavirondian granites in western Kenya by the whole-rock Rb–Sr isochron method We have also reanalysed the samples of Masaba Granite studied by Old and Rex[5], and have obtained very different results from those authors Sample localities are shown on Fig 2

Two distinct phases of magmatism bracket the Kavirondian system The Mumias Granite cuts post-Kavirondian structures in the Kakamega region, and has produced a distinct thermal aureole in the Kavirondian sediments to the north This granite is contiguous with the Kitosh Batholith of Kenya, which becomes the Buteba Granite in Uganda[5] The Migori G2 granite, the only body for which there is good evidence for a pre-Kavirondian age, intrudes the Nyanzian but is nowhere seen in contact with the Kavirondian Its pre-Kavirondian status[11] is based on its petrographic similarity to some of the well-rounded boulders contained within a basal Kavirondian conglomerate

Fig 1 Geological setting of Tanzanian Shield (Inset map after T N Clifford)

overlying the Nyanzian a few kilometres north-east of the G2–Nyanzian contact (Fig 2) The Masaba granite has an uncertain relationship with the Nyanzian and Kavirondian systems, but its eastern boundary seems to truncate Nyanzian–Kavirondian contacts[6]

Although the samples of Migori G2 are petrologically more varied than the very uniform boulders from the Kavirondian conglomerate, KEN 167 and 168 are porphyritic quartz monzonites which are indistinguishable in thin section from the boulders The post-Kavirondian granites, also quartz monzonites, contain abundant biotite or chlorite, and rare hornblende, whereas the pre-Kavirondian samples are characterised by abundant hornblende

Analyses were performed at the Universities of Leeds (Migori G2, Kavirondian boulders, and reanalyses of Masaba), Texas (MA 100–103, analysed by S DeLong) Toronto and Carleton University Concentrations of Rb and Sr were determined by isotopic dilution at Leeds and by X-ray fluorescence elsewhere, with an estimated precision of ± 1 5% (1σ) Strontium isotope ratios have a precision of ± 0 03% on critical samples (Migori G2), and 0 4–0 05% on the remainder Numerous inter-laboratory comparisons showed no systematic differences

Fig. 2 Sample localities Ma, Masaba Granite, Bu, Buteba Granite, Mu, Mumias Granite, Mi, Migori G2, Kc, Kavirondian conglomerate, T, Tertiary volcanics Small Kavirondian outcrops are omitted
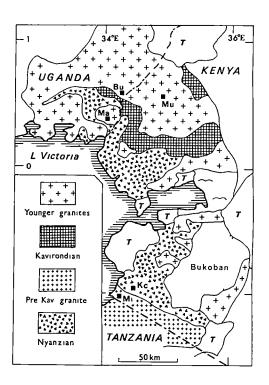
Table 2 Rb–Sr data from granites in north-western part of Tanzanian Craton

	Sample no	Rb (p p m)	Sr (p p m)	$^{87}Rb/^{86}Sr$ (atomic)	$^{87}Sr/^{86}Sr$ (atomic)
	KEN 167	115	807	0 414	0 7181
	KEN 168	123	704	0 508	0 7210
G2 Migori	KEN 169	114	726	0 455	0 7196
Gold Belt	KEN 170	95	839	0 329	0 7143
SW Kenya	KEN 171	110	565	0 570	0 7239
	KEN 172	98	837	0 338	0 7146
	KEN 173	126	848	0 430	0 7186
	KEN 149	101	878	0 335	0 7153
	KEN 150	120	705	0 495	0 7213
Boulders,	KEN 151	112	821	0 396	0 7183
Kavirondian	KEN 152	106	850	0 360	0 7171
conglomerate	KEN 153	139	642	0 630	0 7246
	KEN 155	136	629	0 629	0 7283
	RO 400	64 5	121 3	1 542	0 7539
	RO 401	76 9	105 1	2 120	0 7810
Masaba	RO 402	63 1	36 1	5 07	0 8727
SE Uganda	RO 404	38 0	236	0 463	0 7166
	RO 423	18 4	222	0 240	0 7131
	MA 100	24	281	0 24	0 7116
	MA 101	33	231	0 41	0 7158
	MA 102	70	151	1 34	0 7513
	MA 103	78	113	2 02	0 7727
	KEN 113	223	134	4 89	0 8831
Mumias,	KEN 114	194	123	4 65	0 8731
SW Kenya	KEN 116	306	281	3 19	0 8077
	KEN 117	186	614	0 87	0 7320
	KEN 118	196	631	0 90	0 7339
Buteba,	*RO 405	272	96 9	8 35	0 9954
SE Uganda	RO 406	287	103	8 29	0 9976
	RO 407	233	112	6 15	0 9177

*$^{87}Sr/^{86}Sr$ determined at Leeds, Rb and Sr at Carleton

Analytical data are presented in Table 2 and Figs 3 and 4 The post-Kavirondian samples, including Masaba, give ages of about 2,500 Myr (Fig 3) The main differences between our data on the Masaba granite and those of Old and Rex[5] lie in the Sr isotope ratios, which they apparently overestimated systematically Our new analyses on the original Masaba samples (pre-fixed RO) are consistent with the results obtained by De-Long on MA 100–103 We consider the latter to be the more reliable, the samples were collected specifically for geochronological analysis, and they yield an isochron age of 2,500 ±80 Myr, with an initial $^{87}Sr/^{86}Sr$ (R_i) ratio of 0 7024 ± 0 0013 The other post-Kavirondian granite, Mumias-Buteba, yields an isochron age of 2,530 ± 50 Myr (R_i = 0 701 ± 0 003) consistent with the original data[5] for these samples Deviations from linearity in both isochrons suggest a measure of geological error, and the uncertainties quoted are estimates based on McIntyre's models 2–4 and York's model 1 (ref 12)

Our minimum age for the Kavirondian system, 2,530 ± 50 Myr, is similar to that given previously[4] A maximum age is obtained from the Rb–Sr isochron on Migori G2 (Fig 4), which gives 2,800 ± 120 Myr (R_i = 0 7013 + 0 0007) with no significant geological error The boulders do not yield an isochron, but they plot sufficiently near to the data on G2 to support the hypothesis that they were derived from that body, the discrepancies can reasonably be ascribed to the effects of weathering There are no strong reasons to suspect the isochron age of G2 to be other than the date of emplacement

The Kaapvaal counterpart of the Kavirondian System, the Moodies Group, is intruded by the Kaap Valley Granite, dated at 3,310 ± 40 Myr by the U–Pb method[9] A possible older limit for the Moodies Group, 3,500 ± 200 Myr, is given by an Rb–Sr mineral isochron[10] on a basaltic komatiite from the underlying Onverwacht group Thus, there is an age difference in the range 500 ± 100–1,000 ± 200 Myr (a length of time comparable with the whole Phanerozoic), between the Moodies Group and the Kavirondian System
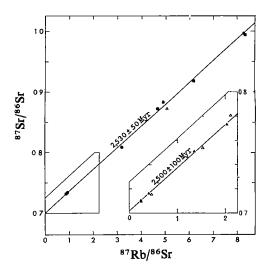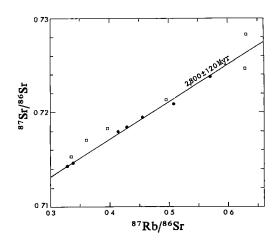
Fig. 3 Rb–Sr isochrons for post-Kavirondian granites ●, Mumias, ■, Buteba, triangles, Masaba (closed symbols used for isochrons) Isochron calculations were made using the method of McIntyre and York[12] Uncertainties are 1σ (67% confidence) Mean square of weighted deviates (MSWD) = 7 2 (Mumias) and 5 5 (Masaba)

No granites older than Migori G2 (2,800 ± 120 Myr) have been found in the Tanzanian Craton[13-15], and nothing unequivocally younger than 2,500 ± 100 Myr In the Kaapvaal Craton the granitic rocks were emplaced over a much longer period, and are divided into two suites on geochemical grounds[16] Granites of the older suite, dated at 3,400–3,000 Myr, can perhaps be considered to be geochemically analogous to the granites of the Tanzanian Craton The 'younger plutons', all of which postdate the greenstone belts, have ages of 2,800–2,500 Myr, but possess no obvious analogues in eastern Africa in the appropriate age range (1,900–2,200 Myr) Further radiometric and geochemical measurements on the granites of the Tanzanian Craton are in progress, and will allow a more precise comparison between the Archaean histories of eastern and southern Africa

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Fig 4 Rb–Sr isochron for pre-Kavirondian granite from Migori, Kenya ●, In situ samples, □, boulders MSWD = 0 8

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Numerical test of Palásti's conjecture on two-dimensional random packing density

RANDOM packing and random space-filling problems have received frequent attention[1-5] In one dimension, this topic is usually called the car-parking problem[6-10] Palásti[11] generalised the parking problem to two dimensions and conjectured that the random packing density in the two-dimensional case is equal to the square of that in the one-dimensional case Here we provide a thorough check on her conjecture and confirm its validity

The procedure of two-dimensional random packing in Palásti's sense is as follows Consider a rectangle T_{xy} with vertices $(0,0)$, $(x,0)$, $(0,y)$, (x,y) where $x,y>1$ Fill the rectangle at random by unit squares whose sides are parallel to the coordinate axes The centre of filling squares is assumed to follow a uniform distribution over the unfilled portion of the rectangle If a unit square overlaps any of the previously placed squares or intersects with the boundary of T_{xy}, then it is discarded The process continues until the remaining space is inadequate for a unit square Let $M(x,y)$ denote the total
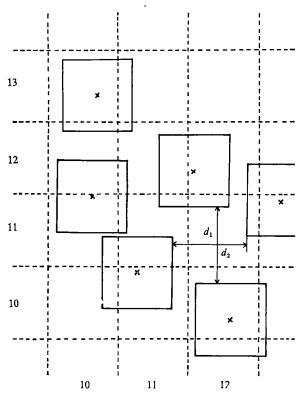
Fig 1 Diagram illustrating the filling procedure Dotted meshes represent unit square sections into which T_{xy} is subdivided Unit squares whose sides are solid lines express cars For example, the sections (10,13) and (11,11) belong to type I and type II, respectively The section (12,11) is of type III since d_1 and d_2 are greater than unity When (12,11) experiences 150 discardings of cars and still remains unfilled, we classify it in type III*

number of unit squares placed in T_{xy} and $m(x,y)$ indicate the expectation of the random variable $M(x,y)$ Palásti[11] proved that there exists

$$\lim_{x\to\infty, y\to\infty} m(x,y)/xy$$

and conjectured that the limit is equal to $c^2 \simeq 0\ 5589$ where

$$c = \int_0^\infty \exp\left[-2\int_0^t (1-e^{-u})/u\, du\right]dt \simeq 0\ 7476$$

This is just the limit of the mean random packing density in one dimension[6] Furthermore, she made Monte Carlo experiments for the four cases of $xy = 5\times15, 10\times15, 15\times15, 20\times15$, which always produced a value of 0 56 Solomon[12] reported similar experiments with results ranging from 0 50 to 0 55 In both cases, however, the size of the rectangle T_{xy} seems too small to test Palásti's conjecture stringently We have devised

Table 1 Computer simulation results for mean packing density

Rectangle T_{xy} (xy)	Lower bound	Present work Upper bound	Present work Mean	s d	Palásti (ref 11)	Previous work Solomon (ref 12) Mean	Previous work Solomon (ref 12) s d
5×15	0 5240 (0 4920*)	0 5347 (0 5080*)	0 5347 (0 5080*)	0 0317 (0 0329*)	0 56†	0 50270	0 02089
10×15	0 5173	0 5327	0 5293	0 0222	0 56†	0 53933	0 02188
15×15	0 5280	0 5462	0 5422	0 0170	0 56†	0 53644	0 01027
20×15	0 5350	0 5450	0 5430	0 0147	0 56†	0 53700	0 01261
20×30	0 5343	0 5495	0 5465	0 0073	—	0 54833†	—
40×40	0 5415	0 5574	0 5536	0 0040	—	—	—
60×60	0 5451	0 5608	0 5571	0 0043	—	—	—
100×100	0 5479	0 5625	0 5593	0 0018	—	—	—

* Another series of ten trials
† Only one trial

a computer program by which a Monte Carlo simulation of this problem can be made even for relatively large T_{xy}

For simplicity we restrict ourselves to the rectangle T_{xy} with x and y integers We subdivide T_{xy} into unit square sections whose number is xy To avoid confusion between a unit square section and a filling unit square, we shall hereafter call the former a 'section' and the latter a 'car' Instead of picking a centre point of a car at random in T_{xy}, choose a section with equal probability and then choose a centre of a car in that section During the course of packing, we classify sections into three categories (Fig 1) A section of type I contains a centre point of a car already placed It is obvious that sections of this sort cannot be occupied by centres of cars again A section of type II is defined as an empty section to which no centres of cars can be assigned In other words, the arrangement of cars inserted in the neighbouring sections prevents the type II section from including a centre point of a car Sections that belong neither to type I nor to type II are termed type III sections At the first stage, all sections are of type III As the procedure goes on, however, some of them change to type I or type II sections Type III sections left unchanged still experience repeated discardings of cars Those sections which have encountered 150 discardings of cars are excluded from the group of type III and renamed type III* sections The iteration proceeds until no sections of type III remain in T_{xy} The number of type I sections gives a lower bound of $M(x,y)$, and the total number of type I and type III* sections yields an upper bound The difference of these bounds was about 1 5% Furthermore, careful examination of type III* sections enables us to evaluate $M(x,y)$ with an error of at most 0 1%

Table 1 shows the results of our computer simulations together with the data of Palasti and Solomon Sample means and standard deviations over ten trials are listed there Our results seem to be in better agreement with those of Solomon than with those of Palasti As the size of T_{xy} increases, the mean packing density approaches $c^2 \simeq 0\ 5589$ When $xy = 100 \times 100$, this value is estimated as 0 5593, which is very close to c^2 Assuming that $M(x,y)$ is asymptotically normally distributed as in the one-dimensional case[9,10], we have a 95% confidence interval of $(0\ 5593 \pm 0\ 0013)$ The validity of Palasti's conjecture is thus confirmed numerically

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Detonation of fuel coolant explosions

In certain circumstances the mixing of a hot liquid and a cooler, vaporisable one leads to an explosive rate of vapour production Such explosions have occured in foundries when molten metals and water mix[1], and when liquid natural gas is spilt on to water[2], they may also occur in liquid-cooled nuclear reactors under accident conditions It seems likely[3,4] that in these events an initial disturbance causes motions which fragment some of the material and so allow rapid heat transfer, this produces

explosive expansion and further fragmentation, and so the reaction propagates through the medium We give here a simple one-dimensional model of a system in which the two liquids are initially coarsely mixed, and show that there is the possibility of an extremely violent thermal explosion, the structure of which is analogous to that of a detonating chemical explosion

Consider a strong shock front progressing steadily through the coarsely mixed material (Fig 1), we assume (and justify below) that close to the front the flow velocities are sufficient to cause fine fragmentation of the hot material and rapid heat transfer The front leaves behind a mixture which attains thermal equilibrium at high pressure, in so doing the material expands and drives the front forward

Applying the equations of conservation of mass, momentum and energy to the material entering and leaving the front[5] we deduce that the possible states (U_2, P_2, V_2) of the material leaving the front are related to the pressure P_1 specific volume V_1 and internal energy U_1 of the material entering the front by

$$\tfrac{1}{2}(P_1 + P_2)(V_1 - V_2) = U_2 - U_1$$

This, together with the equation of state for the material leaving the front $U_2(P_2, V_2)$, defines a unique relationship between the possible values of P_2 and V_2 (called the Hugoniot or shock adiabatic)

From the theory of detonation[5] it can be shown that for a specific set of initial conditions, there is only one state (point O in Fig 2a), for the material behind the front which ensures that the explosion is stable In this state the velocity of the material leaving the front is just sonic with respect to the front—this is called the Chapman–Jouguet (C–J) condition Sonic choking at the C–J plane enables the explosion front to propagate independently of the motion in the region behind it

The explosion propagates with a velocity which is greater than the speed of sound in the medium ahead of the front The pressure and density both rise at the shock front in the 'von Neumann spike' (point N in Fig 2a), and the velocity in the frame of the front falls from the shock velocity to a low value (Fig 1) Behind the shock, as fragmentation and energy transfer occur, the velocity increases, reaching the local speed of sound at the C–J plane Pressure and density both fall in this region, along the straight line of constant mass flux NO in Fig 2a

Behind the C–J plane the material expansion continues The form of the solution in this region is 'self similar', that is the pressure and velocity profiles are always the same shape but their scale increases linearly with time Thus the expansion rates in this region fall inversely with time, and for times much longer than the heat transfer timescale equilibrium will be maintained throughout, and the expansion will be adiabatic

By analogy with the terminology for chemical explosions, we call a thermal interaction which propagates stably behind a shock front a 'detonating thermal explosion' Because the explosion must satisfy the C–J condition, we can predict the propagation velocities and pressures without a detailed knowledge of the fragmentation and energy transfer processes, assuming only that energy transfer is complete at the plane a_1 which the C–J condition is satisfied (see, for example, ref 6) Figure 2b shows the shock adiabatic for a system consisting of equal volumes of tin, water and steam The tangent from the initial point, $P_1 = 1$ bar, $V_1 = 0\ 37$ g cm^{-3}, meets the curve at a C–J point of pressure ~ 1 kbar The propagation velocity

$$v_1 = V_1 \sqrt{[(P_2 - P_1)/(V_1 - V_2)]} \sim 3 \times 10^4 \text{ cm s}^{-1}$$

If there is little or no vapour initially present the Hugoniot curve remains more or less unchanged, but the point from which the tangent is drawn moves to a lower specific volume so that the pressure of the C–J state increases considerably The propagation velocity and the velocity differential causing fragmentation are also increased by the absence of vapour

The model as so far outlined does not demonstrate that a detonation will occur, merely the conditions which it will fulfil if it does To demonstrate the realisability of such an explosion we must consider the fragmentation processes behind the front

In a detonating chemical explosion, the leading edge of the reaction front is a shock in the unreacted material, the compression raises the temperature of the material passing through it to such a level that combustion occurs close behind the shock We consider that the structure of a detonating thermal explosion may be similar, a simplified picture (Fig 1) is that the leading edge of the reaction region is a shock in which the vapour blankets are collapsed causing some degree of mixing and allowing efficient heat transfer to begin The steep pressure gradient at the shock front accelerates the materials entering it at rates dependent on their densities, so causing velocity differentials between the residual vapour, coolant and fuel If these velocity differentials are sufficiently large, fragmentation will occur before velocity equilibrium is re-established Such mechanisms for the breakup of drops have been observed in chemical detonation of two-phase systems where the fuel is in droplet form in a gaseous oxidising atmosphere[7]

We may use the data of Simpkins and Bales[8] on the breakup of liquid drops behind shock fronts to examine this event in more detail They showed that a drop of radius r_0, density ρ' in a flow of velocity u, and density ρ would break up in a time t given by

$$(\rho/\rho')^{\frac{1}{2}}ut/r_0 \simeq 44B_0^{-1/4}$$

Here B_0 is the Bond number, given by
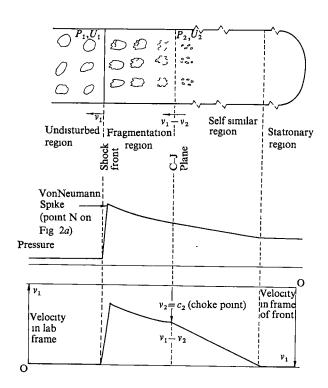
$$B_0 = (\rho'gr^2)/\sigma = \tfrac{3}{8}C_DWe = (3u^2r_0 C_D)/8\sigma$$
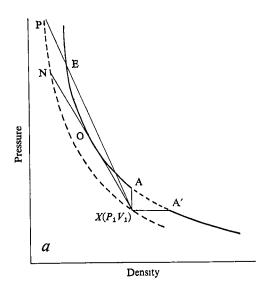
where C_D is an effective drag coefficient (~ 2) and σ is the surface tension of the drop We compare the breakup time t with that required to achieve velocity equilibrium, namely

$$t' \simeq (8/3)(\rho'r_0)/(\rho u C_D)$$

The breakup time must be shorter than this for successful

Fig 1 Geometry and schematic pressure and velocity profiles of a one-dimensional explosion
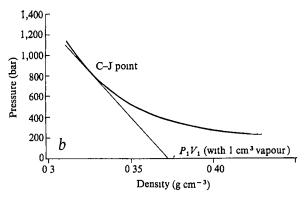
Fig 2 *a*, Schematic shock adiabatic Solid curve, reacted material, dashed curve, unreacted material *b*, Shock adiabatic for an initial mixture of equal volumes of tin at 1,000 °C, water at 100 °C and steam

fragmentation For tin drops of 1 cm diameter in water this condition is satisfied if the Bond number is greater than $\sim 10^4$ The differential velocity, u, is initially greater than v_1-v_2 (Fig 1), where $v_1-v_2 = \sqrt{[(P_2-P_1)(V_1-V_2)]} \sim 10^4$ cm s^{-1} (Fig 2*b*) Thus Bond numbers are greater than 10^5, and breakup by Taylor instability is achieved in typically $\sim 2\times10^{-4}$ s, ~ 10 cm behind the front The liquid drop will break into particles a micrometre or so across ($\rho u^2r/\sigma \sim 8$), and so the time for heat transfer will be much shorter than that for breakup Thus, a short distance behind the front energy transfer will be complete and a homogeneous equilibrium state will be produced These equations, though derived for motion down a long tube, should apply in any explosion if the reaction front is plane on a scale of many times its thickness so that all flow velocities are normal to it Single-phase chemical detonations have reaction fronts which are only a few tenths of a millimetre wide so that detonations can occur even in very small regions without external constraint On the other hand, two-phase detonations, whether chemical or thermal, have relatively long reaction lengths True one-dimensional detonation can only occur in constrained geometries, or regions of coarse intermixing which are large compared with the reaction length

We show now that the experiments so far performed in which large efficiencies have been achieved have approached the conditions necessary for detonating thermal explosions

Consistently high pressures (up to 600 bar) have been observed when columns of water are impacted into molten materials in strong tubes[9] It has been observed that material is excavated to a depth of about one tube width by each successive bounce of the column, and that the largest pressure peak is usually produced on the second impact If material which was

excavated on the first bounce is coarsely distributed over a few tube diameters at the bottom of the column, then the pressure pulse provided on second impact may trigger a detonating thermal explosion in this rather short (~ 5 cm) but roughly homogeneous and constrained medium. Experiments in which longer initial distributions of material were produced would provide a good test of the model.

We have observed explosions of quite high efficiency between Freon-22 and hot water[10]; on a foundry scale efficient interactions in metal–water systems have also been reported. In both cases there is photographic evidence for a degree of intermixing of the two components before explosion, though it is probable that in neither case is the characteristic dimension of the system much larger than the reaction length. Such explosions are thus intermediate in character between those in very constrained geometries and those in unconstrained stratified media.

Experiments in horizontally stratified and unconstrained metal–water mixtures have provided direct evidence for explosion propagation[4]. The reaction region is observed to propagate at only moderate velocity ($\sim 3 \times 10^3$ cm s^{-1}) along the fuel-coolant interface, and pressures of a few tens of bars are generated. These interactions do not fulfil the conditions for a one-dimensional detonating explosion; there is little premixing of tin and water, and the reaction front is necessarily of a size comparable with its width, so that sideways flow out of the front is very important. Accordingly, vapour generation is not suppressed, and the speed of sound in the front will be low. But it seems possible that for reasons of stability similar to those applying to a one-dimensional detonating explosion, the Chapman–Jouguet condition also holds for these two-dimensional explosions. Thus, because of the low speed of sound at the front, material will leave the front rather slowly and so the propagation itself will be slow; the low propagation velocities, and hence low efficiencies, seen in our small-scale experiments may also be characteristic of larger-scale explosions in unconstrained stratified media. But the first slow passage of the reaction front over a pool of metal may not cool all the available metal and could leave behind a coarsely mixed region through which a second, more efficient, explosion could propagate; this is indeed consistent with the photographic evidence of our larger tin–water interactions.

Although further work is necessary for a full quantitative theory of two-dimensional explosions, the present one-dimensional model is applicable to large scale events in, say, nuclear reactors. The SPERT 1D reactor was destroyed by an explosion which occurred when one third of the fuel was melted in an overpower transient. The high fraction of vapour in the core (~ 30%) at the time of the explosion presents difficulties for most explanations of the large pressures generated (~ 250 bar), see ref. 11), but these are easily understood on our model.

Fuel melting could also arise in fast reactors under fault conditions, and our calculations[12] show that pressures of the order of 15 kbar could be produced from large scale events involving sodium and molten uranium dioxide.

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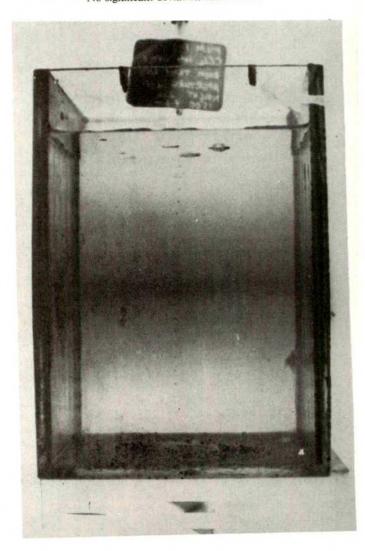
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Fall of liquid metal into water

THERE is a possibility of explosions under a wide variety of conditions[1-6] when liquids are rapidly mixed. When molten tin is poured from a crucible into water in defined conditions, a rapid explosive interaction takes place producing a sponge-like mass of debris[7-9]. We have repeated these experiments using an apparatus which provides small (~ 0.25 g) and reproducible single drops of molten tin, and have been able to determine quantitatively the dynamics of the interaction by a photographic technique. Using a metal temperature of ~ 570 K and water at room temperature metal drops were produced which entered the water in a straight vertical line but were then observed to deviate suddenly and sharply from their course without losing their pear-like shape. This metal temperature was just below that necessary to produce explosive interactions for the given water temperature.

We have photographed the fall of drops of molten tin at 570 K, and mercury and carbon tetrachloride at room temperature, into tap water at room temperature, using stroboscopic illumination. The path of a falling drop is clearly visible, both in the air and below the water, and the velocity may be determined from the image spacing. Although mercury and carbon tetrachloride fall in vertical straight lines to the first order (Fig. 1), molten tin drops

Fig. 1 A carbon tetrachloride drop falling from a height of 2 cm into water. Both liquids were at 293 K, and the drop was photographed in a stroboscopic light at 1,500 flashes min^{-1}. No significant deviation can be seen.
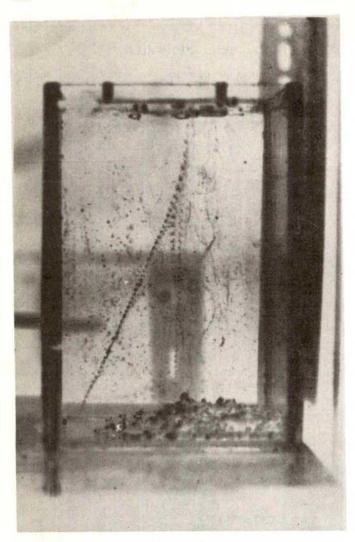
Fig. 2 Molten tin drops at 570 K falling from a height of 23 cm into distilled boiled water at 293 K. The drops were photographed in a stroboscopic light at 9,500 flashes min⁻¹. A significant deviation can be seen.

which takes place between stable film boiling and transition boiling. In less than 0.25 ms the vapour shell is explosively destroyed giving rise to a regime of boiling described as pulsation boiling, which consists of a thin oscillation vapour film. This then progressively changes to nucleate boiling from the front of the sphere to the back, with a pulsating vapour ring separating the nucleate boiling zone from the remaining thin vapour film. If the transplosions or the pulsating vapour rings occur in a similar fashion on molten tin drops, they could perhaps provide an explanation for the considerable momentum found to be imparted during the quenching process.

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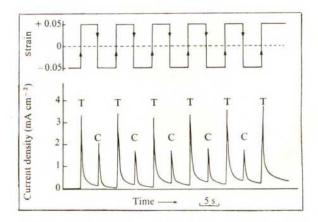
(Fig. 2) initially fall in vertical straight lines, but after a few centimetres in the water deviate from this straight line by up to $\frac{1}{3}$ rad.

It has been suggested[7-9] that fragmentation of the molten tin is linked with the collapse of a vapour blanket surrounding the molten metal during the change from stable film boiling to transition boiling. Using a model for the collapse of a vapour bubble developed by Plesset and Chapman[10], Buchanan and Dullforce[7] predict the velocity and dimensions of a slug of water resulting from the collapse of a bubble of initial radius R_m, which then penetrates the liquid metal initiating the first cycle of a fragmentation process. From our photographs, using simple particle dynamics, it is possible to find the direction and magnitude of momentum imparted to the tin drop. Assuming that this momentum results from the collapse of a single vapour bubble adjacent to the tin drop, R_m can be evaluated. Values of the momentum imparted lay between 4 and 12 g cm s⁻¹, and the momentum was directed upwards at angles to the vertical between 0.87 and 1.22 rad. Corresponding values of R_m were between 0.25 and 0.35 cm. These seem too large for single bubble collapse to be a complete explanation for an initiation of the fragmentation process, at least for molten tin drops of radius 0.15 cm.

Work has been carried out[11] on quenching hot, solid metal spheres by moving them at a constant speed (of a similar value to that of our tin drops) through a tank of water. A phenomenon termed a "transplosion" is described,

Strain enhanced dissolution effects in the corrosion-fatigue failure of metals

IT has been suggested[1] that yield assisted dissolution, which occurs during the corrosion fatigue of high carbon steels in aqueous sodium chloride solutions, may be responsible for the initiation of cracking. Furthermore, it is likely[1] that at low stresses the process of crack initiation largely determines the overall corrosion-fatigue life. It now seems that these significant dissolution effects can be produced and observed in metals subjected to strain cycling in corrosive solutions.

We have studied the anodic dissolution of mild steel, aluminium and stainless steel, during cyclic plastic deformation in aqueous electrolytes, in order to simulate on a macroscopic scale the local conditons that occur at stress concentrations during corrosion fatigue. Specimens held under potentiostatic

Fig. 1 Dissolution transients in 18/8 stainless steel, strain cycled in 3.7 M H_2SO_4 at 1,042 mV (normal hydrogen electrode—n.h.e.)
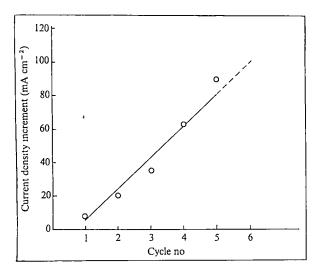
Fig 2 The variation of dissolution transient magnitude with cycle number at a potential close to the active–passive transition, using 18/8 stainless steel in 3 7 M H_2SO_4 at 262 mV Strain, $-0 05$ to $+0 05$

control in the electrolyte were subjected to square wave strains in the range $\pm 0 02$ to $\pm 0 05$ at strain rates of $\sim 0 30$ s^{-1} These strain levels were chosen to provide a sufficient number of slip steps on the surface of the metal to give dissolution effects large enough to be measured In practice, overall strains of this magnitude are unlikely to be encountered even at stress concentrations Even when small local plastic strains occur, however, the emergent slip steps will behave chemically in a similar manner

Dissolution transient currents were observed during both tensile and compressive strain reversals The transients reached peak values during the application of the strain or at the position of maximum strain, and decayed during the constant strain portion of the strain cycle (Fig 1) The magnitude of the transients, their position relative to the strain cycle and their decay characteristics have been found to depend on several factors, including the potential of the metal surface, the magnitude of the strain reversal, the strain rate and the tendency for surface films to form
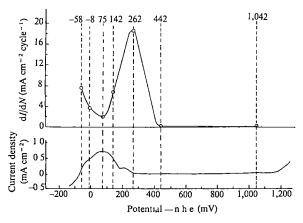
Fig. 3 The change of dI/dN relative to the anodic polarisation curve for 18/8 stainless steel in 3 7 M H_2SO_4

A particularly interesting type of behaviour was observed with 18Cr–8Ni (18/8) stainless steel in 3 7 M H_2SO_4 where repeated strain cycling at active and just passive potentials caused a progressive increase in the magnitude of the tensile dissolution transients The initial transient at all potentials was found to be $< 10 mAcm^{-2}$, at potentials close to the active–passive transition, however, the transients were found to grow by an order of magnitude

within six or seven strain cycles (Fig 2) Assuming that dissolution was concentrated on emergent slip steps at the metal surface these transients represent a local increase of $> 1,000$ in the dissolution rate The rate of increase of the peak value of the current transients per cycle, dI/dN, varies with potential for a cyclic strain of $\pm 0 05$ (Fig 3) It is evident that dI/dN reaches maximum values over a limited potential range coincident with the active–passive transition region, this effect has also been observed at strain levels below $\pm 0 05$ Dissolution effects occurring beyond 10 or 15 cycles have not been investigated because of experimental difficulties in continued cycling at high strain levels It cannot, therefore, be assumed that dI/dN will remain constant during subsequent cycling

If, in corrosion fatigue, stress concentration effects are sufficiently high to induce local plastic deformation on the surface of the metal then the observed dissolution behaviour is likely to occur and could assist crack initiation by a combination of reversed slip and dissolution[2] The evidence suggests that for stainless steel in H_2SO_4 the maximum contribution of dissolution to the initiation of cracking should occur at the active–passive transition Corrosion-fatigue data on 18/8 stainless steel in H_2SO_4 at controlled potential is not available It is interesting to note, however, that Spahn[3] has found the minimum corrosion-fatigue properties of a 13% Cr stainless steel in H_2SO_4 occur at potentials in the active–passive range, that is, at potentials equivalent to those where we observe dI/dN to reach a maximum value

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Tough fibrous composites

GORDON and Jeronimidis[1] and Atkins[2] have noted that increased fracture toughness in fibrous composites can be obtained by an enhanced decoupling of the fibre–matrix interface Gordon[1] has pointed out that in the case of wood (natural cellulose), the decoupling of the 'reinforcing elements' from the rest of the structure is stress dependent and the cells in the vicinity of the fracture face can extend by about 20 per cent before breaking, absorbing a great deal of energy in the process The failing strain of the material as a whole is, however, about 1% Atkins[2] pointed out that increased works of fracture can be obtained in boron fibre expoxy resin composites by arranging for a distribution of strongly and weakly bonded interfacial regions along the fibres During crack propagation the weakly bonded regions allow greater lengths of fractured fibres to be pulled out of the matrix with a concomitant increase in the work of fracture The tensile strength of the composite system is not affected significantly but the overall failing strain is again limited by the failing strain of the reinforcing fibres to about 1%

It has been pointed out[3,4] that the fracture toughness of fibrous composites can be enhanced by a stress controlled decoupling mechanism and, moreover, that fibre fracture can thus be prevented Very large tensile deformations are thus rendered possible as continuous reinforcing elements are pulled through the composite structure The design and behaviour of experimental non-fracturing reinforcing elements has been reported[5-7,12] and the enhanced work of fracture of sheet metal reinforced with such elements has been observed[8-10]

Not only do such systems possess fail-safe characteristics, because of the non-fracturing nature of the reinforcing fibres, but the reinforcing phase is completely insensitive to stress concentrations Because of this, these systems can absorb very much more mechanical energy without fracturing than the toughest work-hardening metal alloys in circumstances where the metal would suffer localised failure[11] That is, of course, the normal situation when sheet metal structures are subjected to localised loading by the impact of a missile or by any other means Prototype systems based on the stress-controlled decoupling principle are under investigation as potential energy absorbing components of motor vehicles (R S Millman and M J Chappell, unpublished)

Studies have been made of the energetics of crack growth in metal plates loaded in tension under fixed grip conditions and reinforced by non-fracturing elements[10] The fracture mechanics of these composite structural systems is quite different from those of other materials because the primary reinforcing phase is completely crack insensitive and also inhibits crack growth in the metal plate Within a specified envelope of conditions, unstable crack growth in the metal plate is not energetically possible, whatever the length of the crack Within these specified conditions the work of fracture of the composite system, calculated in the conventional way, becomes, in effect, infinitely large

It is interesting to speculate whether analogous stress controlled decoupling mechanisms could be made to operate at the molecular level on the cross links between the molecular chains of polymeric systems

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Organochlorine residues in Antarctic snow

DDT is a useful model compound for studying the circulation of a toxic pollutant in the global environment[1,2] An understanding of this process could in future be related to potentially more hazardous materials Present models of the dynamics of DDT circulation can account for only a small fraction of the amounts of DDT and DDE which are known to have been released into the environment Major unknowns include the extent to which the atmosphere and oceans act as reservoirs and the transfer rate of these residues from the atmosphere to the oceans where, according to present ideas, they may be removed from circulation by transfer to the abyss[3] Such atmospheric and oceanic transport mechanisms may carry pollutants into the ecologically protected area of Antarctica and it is necessary to assess the extent to which this is occurring and the relative importance of alternative input routes The atmosphere has been assumed to play the major role in the transport cycle but there is a lack of supporting data We report here levels of DDT and metabolites

in Antarctic snow which suggest that the role of the atmosphere in the transport of DDT may have been overemphasised

Measurements of global background levels are hampered by contamination problems at the very low concentrations found[4], by the proximity of local sources of emission, and by interference from other more dominant organic components (as in oceanic measurements) Studies in the interior of Antarctica largely overcome the last two problems Subzero temperatures throughout the year combined with negligible biological activity ensure minimal chemical alteration or diffusion of trapped pollutants after deposition of the snow The year and season of deposition of a particular snow sample may be established by investigation of the stratigraphy of the snow section from which it was taken The present study forms part of an attempt to establish upper limits of concentration of organochlorine residues in contemporary Antarctic precipitation

Representative snow samples from the previous 5–10-yr accumulation were collected during December 1969 on a journey of 450 km inland from the British Antarctic Survey's Halley Bay station (75°31'S, 26°42'W) Sampling procedures were designed to reduce contamination to a minimum by repeatedly trimming large snow blocks before packing them for transport The snow was returned to England in the frozen state, wrapped in pre-cleaned aluminium foil and sealed in steel containers

Samples for analysis were obtained by continued trimming of the snow blocks in a glove box under purified nitrogen at -10 °C Analyses were carried out in a closed glass system[13] under purified nitrogen The system allowed melting, meltwater transfer, and extraction and concentration steps to be carried out in sequence so that at no stage did the samples contact a laboratory atmosphere Continuous cyclic liquid–liquid extraction was used to transfer the organochlorine components in 1 l aliquots of meltwater into 20 ml hexane The hexane solution was subsequently concentrated to 20 µl Meltwater samples, to which had been added radiolabelled pp'DDT at the 1×10^{-12} g g^{-1} level, were used to check the efficiency ($>90\%$) of these procedures Extracted components were identified without cleanup using a Pye 104 gas chromatograph with electron capture detection and 3 m × 3 mm glass columns packed with 1 5% OV-17/QF-1 and 2% SE-30 on 100–200 mesh Chromosorb G Identification of DDT was confirmed by treating the extract with alkali, which eliminated the DDT peak and enhanced the DDE peak Detection limits, which were imposed by incidental contamination, were 3×10^{-14} g g^{-1} water for both DDT and DDE

The data reported here were obtained from 10 snow samples representative of accumulation laid down between 1965 and 1969 at two sites, 360 and 400 km from the coast, where possible interference from oceanic components was minimal Results from two duplicated samples agreed within experimental error (10% at 1×10^{-13} g g^{-1} to 50% at 2×10^{-14} g g^{-1}) Peaks coincident with pp'DDT and pp'DDE occurred in all samples studied The total concentration of DDT and its metabolites (DDT-R) ranged from 0 1 to 2 0 × 10^{-12} g g^{-1}, however pp'DDE was consistently more abundant than pp'DDT by a factor of 1 3 to 1 8 There was no evidence for families of other components corresponding to polychlorobiphenyls (PCBs) in the 50–60% chlorine composition range, which are widespread in the northern oceanic areas[5] and have been reported in birds which range north of the Antarctic Convergence[6]

Our data contrast with earlier measurements[7] made on snow from Plateau Station, Antarctica where some of the samples were reported to contain 40×10^{-12} g g^{-1} of pp'DDT alone These figures are comparable with average measurements made in England over the same period[8] It is evident that studies more widely based both in time and space are required in Antarctica

Higher levels of DDE than DDT have also been reported in some English rainwater samples[8], particularly outside the spraying season when DDT levels were relatively low This could be explained simply by evaporation of the more volatile metabolite DDE from deposited DDT which has undergone microbial degradation The increased DDE/DDT ratio indicated by the Antarctic samples could point to further photodegradation of

DDT vapour in sunlight[9] during atmospheric transport The very low background levels add further weight to Cramer's[2] suggestion that perhaps too much emphasis was placed on the atmosphere as a reservoir and transporter of DDT in an earlier model described by Woodwell[1] in which 60×10^{-12} g g^{-1} was considered to be a global average for precipitation Further sources of chemical[14] and biological degradation must be sought

Previous studies[6,10-12] of animal species which remain closely associated with the Antarctic continent throughout their life cycle (Emperor penguins, Adelie penguins, crabeater seals and snow petrels) have yielded lipid analyses of total DDT-R ranging from 0 to 200×10^{-9} g g^{-1} The average content of all samples so far studied is approximately 90×10^{-9} g g^{-1} Taking an initial value of 1×10^{-12} g g^{-1} DDT-R in Antarctic precipitation, an enrichment factor (concentration in animal/concentration in environment) of about 90,000 is obtained This is compatible with values found elsewhere in the environment[3] The implication is that the animal species which remain close to the Antarctic continent are in equilibrium with the surrounding air and natural water It is not necessary to invoke a passage up the food chain of pesticide residues from oceanic nutrients drawn southwards across the Antarctic Convergence

The extremely low levels of PCBs in our snow samples (5×10^{-14} g g^{-1}) agree with data[8] from Adelie penguins and snow petrels, in which the ratio of DDT-R/PCBs is much greater than that found in species which range north of the Antarctic Convergence, despite the marked increase in DDT-R in these species[6,12] These results indicate that the Antarctic environment is much more strongly shielded from penetration by PCBs than by DDT-R This would tend to argue against the idea that, at least on a global scale, the atmosphere is the predominant mode of transport for PCBs

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Mutation affecting taste perception in *Drosophila melanogaster*

ANALYSIS of mutant organisms can elucidate by contrast the development of the normal phenotype and this approach can be used to break down behavioural characteristics into their embryological and physiological components Taste in insects is a characteristic suitable for such a study Genetic analysis of taste in *Drosophila melanogaster* can complement the electrophysiological and anatomical information on taste organs of flies obtained mainly from *Calliphora* and *Phormia* The taste organs of flies are located predominantly on the labella at the tip of the proboscis, and on the tarsi On each half labellum of *Drosophila* there are 36–42 taste bristles on the surface and some 25 taste pegs between the pseudotracheae All but five or six bristles are penetrated by the dendrites of four chemoreceptors (the remaining ones having two dendrites), while there is only one chemoreceptor in each interpseudo-

tracheal peg Each taste organ is also provided with a mechano-receptor neurone (R. F , Bleiser-Avivi and J A , in preparation) Of the four dendrites penetrating to the pore at the tip of the taste bristles in *Phormia*[1-3], one is a water sensor, one reacts to sugars and two react to salts[4-6]

The normal fruit fly responds to stimulation of its taste organs by a vigorous extension (or retraction) of the proboscis which may be followed by consumption of the solution offered A standard testing procedure was developed 4-5-d-old flies were put into funnels covered with a nylon net, they were starved and desiccated for 3 h at 28 °C and then the funnels with the flies were transferred to Petri dishes containing a cotton wool pad soaked with a test solution to which a red food colouring was added The flies were allowed to consume the solution through the net for 20 min (at 28 °C) The proportion of flies with red coloured intestines expressed the reaction of the flies to the taste of the solution Wild type *Drosophila* consume a 0 1 M sucrose solution but reject it when 1 M NaCl is added
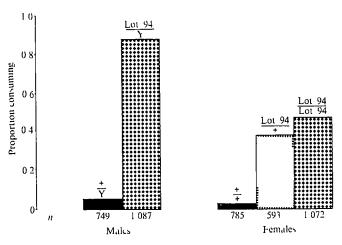
Fig 1 Proportion of flies consuming the test solution (1 M NaCl+0 1 M sucrose) under standard conditions 4-5-d-old flies, 3 h starvation and desiccation at 28 °C, 20 min feeding at 28 °C +, The normal allele of the Lot-94 salt-tolerant mutation (in the heterozygous females it was carried over the In(1)FM6, $y^{31d} sc^8 dm B$ chromosome) *n*, Number of flies tested

Mutations were induced by feeding young males of the wild-type Qiryat-Anavim (QA) stock with a 0 2% ethylmethanesulphonate +1% sucrose solution for 24 h The treated males were mated to yellow, attached-X females F_1 progeny were scored for salt tasting with a standard screening solution of 1 M NaCl +0 1 M sucrose About 3% of wild-type males consume this solution normally All males with red intestines were mated individually to attached-X females and cultures in which a high proportion of the male progeny consumed the screening solution were maintained The suspected X-chromosome mutations to tolerance to the taste of salt were designated 'Lot'[7] Three Lot mutations have been characterised so far[8] One, Lot-94, was mapped to the left of *f*, at 55 5–56 0 on the X-chromosome (J A and Baker, in the press) Homozygous females of this mutation were less insensitive to NaCl solutions than the mutant males This might be only partly the result of the generally greater reluctance of females to consume any solution under our experimental conditions No clear conclusion on the dominance of Lot-94 could be reached, since in some heterozygous combinations it reacted like a partial-dominant mutation (Fig 1), while in others it was completely recessive (see controls in Table 2) Another mutation, Lot-114, probably allelic to Lot-94, was completely recessive in all combinations tested The third mutation, Lot-235, is a recessive mutation at another locus

Response to labellar and tarsal stimulation was tested separately to find out whether the Lot-94 mutation affected both sites QA and Lot-94 flies were fixed in rows on a double-

Table 1 Response of wild-type (QA) and mutant (Lot-94) males to 0 1 M sucrose (Suc) and to 1 M NaCl+0 1 M sucrose (Mix)

a, Proportion of flies extending proboscis when solution was applied up to three times

Applied to	QA Suc	Mix	Lot-94 Suc	Mix
Labellum	0 91±0 08	0 69±0 08	0 91±0 11	0 77±0 19
Tarsus	0 64±0 16	0 31±0 12	0 72±0 13	0 16±0 10

Each proportion is based on 150–220 flies
t-tests QA against Lot-94 Labellum, Suc $t = 0$, Mix $t = 0$ 97 Tarsi, Suc $t = 1$ 14, Mix $t = 2$ 90, $P < 0$ 01

b, Duration (s) of consumption in flies that extended proboscis

Expt	QA Suc No of flies	Duration (s)	Mix No of flies	Duration (s)	Lot-94 Suc No of flies	Duration (s)	Mix No of flies	Duration (s)
1	14 3	>30 18 7	16	4 8± 3 3	14 3	>30 24 0	17	13 4± 8 0
2	18 5	>30 26 2	21	5 1±5 2	17 4	>30 27 8	22	16 9±11 3
3	25 6	>30 24 7	25	5.2±4 7	20 12	>30 21 8	30	16 8± 9 4

Maximum feeding time was 30 s

Table 2 Salt-tolerant gynandromorphic flies

Organ	Proportion of flies Both sides non-mutant	One side non-mutant one side mutant	Both sides mutant	Fate map distances* Af	ff′
Antenna	0 20	0 52	0 79	28 2	0 2
Head	0 20	0 56	0 76	29 5	0 5
Eye	0 19	0 54	0 76	30 2	1 7
Proboscis	0 23	0 43	0 67	31 2	12 0
Wing	0 16	0 54	0 72	34 7	4 6
Leg 1	0 15	0 52	0 71	33 1	10 6
Leg 2	0 16	0 56	0 70	35 0	5 0
Leg 3	0 22	0 54	0 70	35 3	2 8
Tergites 2 and 3	0 27	0 52	0 63	38 6	5 4
Non-gynandromorphic sibs (controls)	0 09		0 86		

The proportion of 532 gynandromorphic flies that consumed 1 M NaCl+0 1 M sucrose solution under standard experimental conditions is given, and the distance in sturt units between the focus of Lot-94 and the reference foci (Af) and between left and right foci of Lot-94 (ff′) was calculated according to Hotta and Benzer[12], assuming that the mutant focus is submissive to the normal focus
*Fate map distances change slightly and proportionally if corrected for consumption of non-gynandromorphic siblings

sided tape on a microscope slide Solution of sugar or mixed salt and sugar was applied from a capillary to alternating flies in the row, by touching their labellum or tarsi The proportion of flies that extended their proboscis was registered In other experiments, the labellum was touched and the length of time that the flies consumed the solutions was recorded (Table 1) There were no differences in the response to sugar between QA and Lot-94 when either the labellum or the tarsi were stimulated Lot-94 flies were significantly less sensitive to salt when consumption time was measured Reduced salt sensitivity was also indicated by extension of the proboscis on touching the labellum with salt solution On the other hand, touching the tarsi with salt solution elicited less proboscis extension in the Lot-94 than in the wild-type QA flies This indicates that tarsal reaction is not the decisive component of the flies' response in our standard testing procedure Getting and Steinhardt[9] postulated the existence of different intermediate neurones for the labellar and tarsal taste receptors in *Phormia* The difference in labellar and tarsal response to salt in the mutant Lot-94 of *Drosophila* is consistent with this finding

The behavioural pattern of a genetic mosaic depends on whether the genotype of the focus essential for the relevant phenotype is mutant or normal In order to produce mosaics for Lot-94, females heterozygous for the unstable ring X-chromosome R(1)2, w^{vc} were mated to y w sn $Lot-94/y^+Y$ males[10] In 25% of these heterozygous embryos, the unstable X-chromosome was lost in an early cleavage division, turning them into XX/XO gynandromorphs Yellow cuticle (y), white eyes (w), singed bristles (sn), sex combs and male genitalia served to detect sectors of XO cells These sectors were also hemizygous for the Lot-94 mutation Since less than half of the gynandromorphs were mutant with respect to their taste response, the mutant focus was assumed to be submissive to

the normal The focus of the mutant was located in the gynandromorphs on the basis of the assumption that the closer a cell was to the focus in the blastoderm, the higher the probability of agreement between their genotypes[11,12] The focus of Lot-94 was closer to that of the antenna than to that of the proboscis (Table 2) The only significant maximum likelihood estimate[13] obtained so far, places the focus 31 sturts (see ref 12 for definition) away from the proboscis and 6 sturts from the midline This does not exclude the possibility that the focus of Lot-94 for taste perception of the labellum is located at a different site from that of the tarsi The focus of Lot-94 was tentatively located midventrally, in the area of the blastoderm giving rise to the brain Thus Lot-94 is a mutation of a perception centre, affecting the recognition of salt, with no detectable effect on that of sugar

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Iridovirus and cytoplasmic polyhedrosis virus in the freshwater daphnid *Simocephalus expinosus*

AN iridovirus and a cytoplasmic polyhedrosis virus have been found as the cause of disease in the freshwater daphnid, *Simocephalus expinosus*, collected in Florida (Fig. 1). Although both types of virus were originally reported from insects[1-3] and are now well known insect virus types[4], neither has been reported before from the Crustacea. Iridoviruses have been reported from other invertebrates and from some vertebrates[5], however, cytoplasmic polyhedrosis viruses have been reported only from insects.

Daphnids infected with iridovirus were first collected during April 1972 from two woodland ponds, approximately 7 miles apart, near Gainesville, Florida. One pond was sampled periodically during each season of 1972, 1973 and 1974. *S. expinosus* seemed to be present only during the spring. During each sampling period, infected specimens were collected until the middle of June, after which host populations declined. The incidence of infected daphnids was never greater than 1%. All specimens were examined against a black background with a fluorescent light. Frankly infected daphnids were opalescent while apparently healthy specimens were grey and translucent.

For electron microscope studies, daphnids were dissected and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer for 3 h, postfixed in 1% osmium tetroxide for 2 h, dehydrated, and embedded in an Epon–Araldite mixture, using standard methods. Examination of ultrathin sections of daphnids infected with iridovirus revealed virions throughout the adipose and glandular tissues, in the epidermis and associated endocuticle, and occasionally in nerve tissue. No virions were observed in muscle or gut epithelial tissues. Virus morphogenesis was restricted to the cytoplasm of infected cells. There was marked degradation of cellular organelles in advanced stages of the disease. Although large numbers of virions accumulated in the cytoplasm of many infected cells, paracrystalline arrays of virions, common in many iridovirus diseases, were not observed.

Structurally, virions were icosahedral and morphologically very similar to other virions of the iridovirus group[5]. Cross sections through mature virions indicated a dense central core (diameter about 96 nm), surrounded by at least one unit-membrane envelope which in turn was surrounded by the capsid. In most virions an amorphous layer of approximately 12 nm was observed between the envelope and the dense central core. The edge-to-edge and point-to-point diameters of mature virions were about 136 nm and 154 nm, respectively. From these measurements, an average fivefold rotational axis diameter of about 140 nm was calculated, using the icosahedral virus model of Mattern[6]. The virus tentatively has been designated iridovirus type 20, conforming with the interim nomenclature system for iridoviruses proposed by Tinsley and Kelley[6].

The cytoplasmic polyhedrosis virus (CPV) was discovered during ultrastructural examinations of daphnids infected with iridovirus. This virus was only observed in the cytoplasm of the midgut epithelial cells. The occlusion bodies were pleiomorphic,
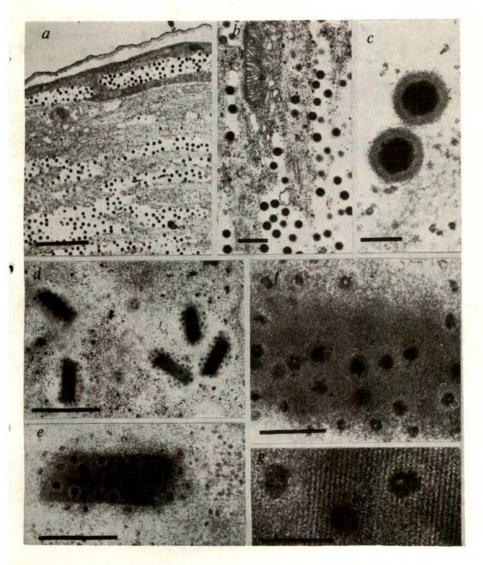
Fig. 1 Electron micrographs demonstrating iridovirus (*a–c*) in a portion of the postabdomen and cytoplasmic polyhedrosis virus (*d–g*) in midgut (caecum) epithelial cells of the daphnid *Simocephalus expinosus*. *a*, Virions in epidermal and adipose tissue (bar=3 μm); *b*, virions in the cytoplasm of an adipose cell (bar=500 nm); *c*, section through two mature virions illustrating the dense core and unit membrane interior to the capsid (bar=100 nm); *d*, oblong polyhedral occlusion bodies in the cytoplasm of a cell (bar=1 μm); *e* and *f*, developing occlusion bodies. Note the virions with electron translucent centres toward the periphery of the occlusion bodies (*e*, bar=400 nm; *f*, bar=200 nm). *g*, Virion cores in an occlusion body (bar=100 nm). Note the crystalline lattice of the occlusion body in (*f*) and (*g*).

the smaller ones ($900 \times 300 \times 300$ nm) being brick-shaped polyhedra while the larger forms (up to 2 μm) were spherical. Virion morphogenesis was different in that unlike other CPVs the virions apparently matured only in close proximity to developing occlusion bodies. Unoccluded virions were frequently observed with electron translucent centres. Occluded virions near the periphery of developing occlusion bodies also occasionally contained electron translucent centres, whereas those occluded within the central areas of large occlusion bodies had uniformly dense cores typical of unoccluded and occluded virions in other CPVs. The virions were icosahedral and approximately 60 nm in diameter. Because the capsid and occlusion body protein were similar in electron density and could not be easily distinguished, this figure should be considered tentative. The virion cores, which were quite distinct, were about 38 nm in diameter.

Couch[8] reported a baculovirus, another type of virus found commonly in insects, in the pink shrimp, *Penaeus duorarum*. The observations reported here, in conjunction with Couch's report, demonstrate that virus types generally considered to be insect viruses, namely, baculoviruses, cytoplasmic polyhedrosis viruses, and iridoviruses, also have as their natural hosts members of the Crustacea. This is not to imply that insect viruses infect Crustacea, but rather that these virus groups have a broader taxonomic distribution than is generally realised. These findings indicate the need for further research directed toward determining the kinds of viruses which occur in Crustacea and other invertebrates. This is important especially because several pathogenic insect viruses are being considered as biological control agents. Knowledge of the distribution of virus types and their specificity in invertebrates will provide valuable background information for the implementation of biological control programmes involving insect viruses.

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Enzyme synergistic for insect viruses

A GRANULOSIS virus (GV) and a nuclear-polyhedrosis virus (NPV) of the armyworm, *Pseudaletia unipuncta*, form a synergistic association in which the infection of the NPV is enhanced[1]. The synergistic factor responsible for the enhancement (56-fold) occurs in the protein of the inclusion body (capsule) that surrounds the particle of GV[2,3]. This factor is present in detectable quantities in one strain of GV (Hawaiian) but not in another (Oregonian)[4,5]. It has been purified by Sephadex gel filtration and seems to be a simple or conjugated protein[3]. We now report that the synergistic factor has properties of an enzyme and can enhance not only the NPV and GV of the armyworm but also other insect viruses. Earlier studies[1,2] had shown that the synergistic factor was heat labile. It became inactive when heated at 85 °C for 10 min (ref. 1). The viral activity, on the other hand, was destroyed at 75 °C for 10 min.

The synergistic factor seemed to be an enzyme because

it catalysed the hydrolysis of *p*-nitrophenyl esters of fatty acids and was inhibited by metallic ions. Tests for hydrolysis of the soluble substrates (esters of two to four carbon atoms) were made in a mixture containing the synergistic factor at 1 mg ml⁻¹, 5% *iso*propyl alcohol, 0.5 mM of the *p*-nitrophenyl ester, and 0.0025 M HCl-Tris buffer, pH 9.0 the pH of optimal hydrolysis. The mixture was held at 25 °C for 30 min, and the amount of product released was measured in the spectrophotometer at 410 nm, the wavelength of the peak absorption for *p*-nitrophenol. In the case of the insoluble substrates (esters of five or more carbon atoms), gum arabic at a final concentration of 1% was added as an emulsifier to stabilise the suspension. The mixture was agitated for 3 min in a sonicator. Subsequently, the treatment was essentially the same as for the soluble substrates, except that after incubation at 25 °C, chloroform was added, and the mixture was shaken for 3 min and centrifuged at 3,000 r.p.m. for 15 min to remove the residual insoluble substrate. The supernatant was collected and read at 410 nm. The controls, which consisted of the esters of the fatty acids, were examined concurrently with the treatments containing the synergistic factor.
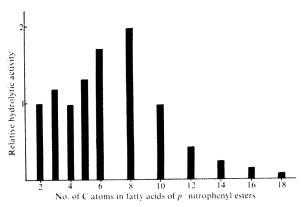
Fig. 1 Hydrolysis of fatty acids by the synergistic factor of a granulosis virus. The hydrolysis of *p*-nitrophenyl acetate is given a value of one. The synergistic factor was isolated and purified as follows. The GV capsules were dissolved largely by treatment for 3 h with 0.02 M NaOH at 4 °C, after which the insoluble material was removed by centrifugation at 20,000 r.p.m. for 1 h. The supernatant was dialysed against 0.01 M phosphate buffer, pH 8.0 and then concentrated in a collodion bag under reduced pressure at 4° C. The concentrate was fractionated by filtration through a Sephadex G-200 gel at 4 °C, and the various components separated were examined with a Beckman DB-GT spectrophotometer at 280 nm. There were six peaks, and each was tested on fifth-instar armyworm larvae for its enhancing capacity as before[2]. The synergistic factor occurred in the second peak fraction, which was concentrated at 4 °C in a collodion bag under reduced pressure and dialysed at 4 °C against 0.001 M phosphate buffer, pH 8, for 16 h. The dialysate was applied to a 1.5 × 5 cm hydroxylapatite column which had been equilibrated with 0.001 M phosphate buffer. The column was washed with 0.001 M phosphate buffer and the synergistic factor was eluted with 0.005 M phosphate buffer, pH 8. The eluate gave a single protein band in disc acrylamide gel electrophoresis using a 7.5% gel in a medium of 8 M urea and glycine buffer, pH 8.7.

In the controls, hydrolysis of the esters of fatty acids occurred also, but at a slower rate than in the presence of the synergistic factor. The results given in Fig. 1 are the differences between the controls and those obtained with the synergistic factor. The relative activity (with *p*-nitrophenyl acetate arbitrarily given a value of 1) of the synergistic factor increased as the carbon atoms in the chains of the fatty acids increased from two to eight, but possibly with a slight dip in activity with butyrate (four carbons). There was a gradual reduction in the hydrolysis of esters with 10 to 18 carbon atoms. Accordingly the synergistic factor catalyses the hydrolysis of synthetic esters of fatty acids.
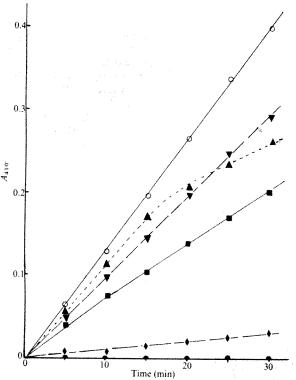
Fig. 2 Effect of metallic ions on the hydrolysis of p-nitrophenyl butyrate by the synergistic factor of a granulosis virus. ○, Control; ▼, Mn; ▲, Mg; ■, Ca; ◆, Cu; ●, Hg.

The optimum pH for the hydrolysis of p-nitrophenyl caprylate by the synergistic factor was studied at various pH using the following buffers: 0.05 M phosphate buffer for pH 6.0, 7.0 and 7.5; 0.05 M Tris-HCl buffer for pH 8.0, 8.5, and 9.0; 0.05 M carbonate buffer for pH 9.5, 10.0, and 11.0. The quantity of released p-nitrophenol was measured in the spectrophotometer at 410 nm. The peak in hydrolytic activity occurred at pH 9.

Since enzymes are often inhibited by metallic ions, the synergistic factor was exposed to five different salts of metals and its activity on p-nitrophenyl butyrate was examined. The salts were $MnCl_2$, $HgCl_2$, $CuSO_4$, $CaCl_2$ and $MgCl_2$, at 1 mM. These metals inhibited to varying degrees the hydrolysis of the butyrate by the synergistic factor (Fig. 2). Copper markedly inhibited and mercury completely inhibited hydrolysis.

The synergistic factor after exposure to these five metallic ions was tested for its enhancing capacity on NPV in the armyworm. After treatment with mercury, the enhancing capacity was destroyed; however, treatment with other metals had little or no effect on enhancement.

Before the isolation of the synergistic factor, our tests indicated that the GV was synergistic for the NPV of the armyworm but not for other insect viruses. But, when the synergistic factor was purified and concentrated and fed together with the GV (Hawaiian and Oregonian strains) of the armyworm, with the NPV and GV of the cabbage looper (*Trichoplusia ni*), and of the beet armyworm (*Spodoptera exigua*), it enhanced the infection of these viruses.

The inclusion bodies, such as the polyhedra of the NPV and of the cytoplasmic polyhedrosis virus (CPV), the capsules of GV, and spherules of poxvirus, are generally believed to protect the virus particles occluded within them from the adverse effects of the environment. The presence of the synergistic factor within the capsule of the GV suggests another function, that of enhancing the infection of the virus.

Although the shapes of inclusion bodies, such as the polyhedra of NPV[6-9] and of CPV[8,10-13] and the capsule of GV[14], are apparently determined by the virus, the origin of these proteins, whether virus- or host-directed, has not been established. The origin of the enhancing enzyme (synergistic factor) is still to be determined, but its detectable presence only in the Hawaiian GV strain suggests that it is a virus-directed protein*.

Recently, the use of chemical insecticides has been encouraged less than before, primarily because of pollution of the environment, the development of resistance in insect pests, the adverse effect on insect parasites and predators, and the conversion of minor pests to major economic pests. One of the areas receiving increasing interest is microbial control where pathogens, such as viruses, bacteria, fungi and protozoa, are used for the control of insect pests. In as much as the synergistic factor enhances not only the NPV of the armyworm but also other insect viruses, it is potentially important in microbial control of insect pests.

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Experimental viral labyrinthitis

CONGENITAL and acquired deafness and acute and chronic vertigo are often attributed to viral infections of the inner ear. Direct demonstration of natural or experimental infection of the membranous labyrinths of the cochlear and vestibular systems has not been practicable, however, because these structures are encased in the dense temporal bones. Evidence that viral infections can cause deafness and vertigo in man is based largely on epidemiological observations and on a limited number of temporal bones examined months or years after the acute event. Temporal bones from patients with deafness associated with mumps, measles, cytomegalovirus and rubella have shown a cochlear–saccular degeneration suggesting that endolymphatic structures may have been infected[1-4]. Except for cytomegalovirus infections, which cause characteristic histopathological changes[4], there are no previous studies of acute viral infections of the inner ear in patients or experimental animals.

We have now studied the pathogenesis of acute viral infections of the inner ear in newborn hamsters, whose temporal bones are not calcified at birth. This made possible the use of fluorescence microscopy to localise infection at a cellular level after direct intralabyrinthine or intracerebral viral inoculations. The distribution of inocula was first localised by the injection of India ink. Following intracerebral inoculation, carbon

particles reached the cochlear scala tympani from the subarachnoid space through the cochlear aqueduct, which is patent in rodents[6]. Carbon particles were seen in perilymph but not endolymph. A few particles also reached the perineural and perivascular spaces of the cochlear modiolus through the internal auditory canal. Direct intralabyrinthine inoculations were performed percutaneously using a microsyringe with a 30-gauge needle. Volumes of 0.005 ml could consistently be introduced through the temporal cartilage into the labyrinth. Injections of India ink showed that carbon particles were variably distributed into the endolymph, perilymph or both.

Three viruses were studied. The mouse-adapted neurotropic (NWS) strain of influenza A virus[7], prepared as a 10% homogenate of infected mouse brains, had an infectivity of $10^{5.6}$—50% lethal doses (LD_{50}) per ml determined by intracerebral inoculation of newborn hamsters. The hamster-adapted Kilham strain of mumps virus[8], grown in Vero cells, had an infectivity of $10^{6.2}$—50% tissue culture doses (TCD_{50}) per ml. The hamster-adapted neurotropic strain of measles virus (HNT)[9], prepared as a 10% homogenate of infected suckling hamster brains, had an infectivity of $10^{6.3}$—LD_{50} per ml determined by intracerebral inoculation of newborn hamsters. Virus pools were diluted 1 : 10 in balanced salt solution, and 0.005 ml of these dilutions was inoculated into each labyrinth or 0.02 ml

was inoculated intracerebrally.

30 min. Rhodamine was used as a counterstain. After final washings, the sections were mounted in neutral glycerol and examined by fluorescence microscopy.

Influenza A virus inoculated by either intracerebral or intralabyrinthine routes resulted in a similar pattern of infection. Immunofluorescent staining demonstrated viral antigen in the loose mesenchymal cells of the cochlear aqueduct and scala tympani 1–4 d after inoculation (Fig. 1a). Eighth nerve ganglion cells in the cochlear (spiral) ganglia and vestibular (Scarpas) ganglia also showed specific fluorescence. No viral antigen was found in endolymphatic structures.

In contrast, mumps virus infected endolymphatic structures. This infection was most consistent after intralabyrinthine inoculation but occasionally some endolymphatic cells contained antigen after intracerebral inoculation. Nine to 12 d after inoculation, cells in both layers of Reisner's membrane, the stria vascularis, and the organ of Corti contained mumps virus antigen (Fig. 1b). Specific fluorescence was also seen in cells of the macula of the saccule and adjacent endolymphatic membrane. Again neurones in both the cochlear and vestibular ganglia stained specifically, but no viral antigen was seen in perilymphatic structures.

Measles virus inoculated by either route infected both
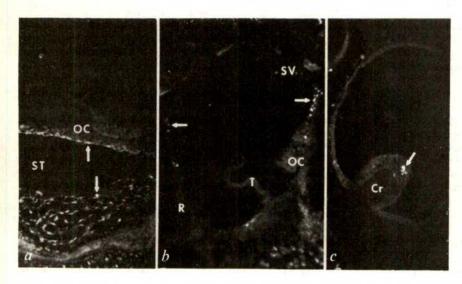
Fig. 1 Micrographs of hamster inner ears stained with fluorescent antibodies. *a*, Cochlea 1 d after intracerebral inoculation of influenza A virus. The arrows indicate the location of specific fluorescence in the loose mesenchymal cells of the perilymphatic scala tympani (ST) below the immature organ of Corti (OC). ($\times$ 78). *b*, Cochlea 11 d after intralabyrinthine inoculation of mumps virus. The arrows indicate the location of viral antigen in the organ of Corti (OC), stria vascularis (SV), and Reisner's membrane (R), but not in the tectorial membrane (T) ($\times$ 78). *c*, Semi-circular canal 3 d after intralabyrinthine inoculation of measles virus. The arrow shows viral antigen in the crista (Cr) ($\times$ 78).

Newborn hamsters were observed for signs of illness following intracerebral or intralabyrinthine viral inoculation. Since hamsters do not have functioning inner ears before day 20 (ref. 10), no clinical signs of inner ear disturbance could be observed. Animals inoculated by either route also developed encephalitis 3–4 d after inoculation of influenza or measles virus and 9–13 d after inoculation of mumps virus. Litters inoculated with diluent remained well and were killed at appropriate times for controls.

After the animals had been killed, optimal preservation of inner ear structure and viral antigens was achieved by using saline perfusion followed by perfusion of cold 95% ethanol. The skinned head, with jaw removed, was fixed in 95% ethanol for 24 h at 4 °C, dehydrated in graded steps of cold 100% ethanol, cleared in cold xylene and embedded in paraffin wax according to the method of Sainte-Marie[11]. Serial horizontal sections of the head including both inner ears were cut on a rotary microtome. At various levels of the inner ears adjacent sections were taken for histological examination and immunofluorescence studies. The paraffin sections were stored at 4 °C until processed for fluorescent antibody staining. The paraffin was removed by washing in consecutive cold xylene, alcohol and saline baths. Sections were then overlayed with virus-specific guinea pig antisera or with control non-immune sera for 30 min. Sections were then washed in saline and overlayed with fluorescein-conjugated rabbit anti-guinea pig γ globulin for

perilymphatic cells and cells in the organ of Corti and crista. Three and four days after inoculation, loose mesenchymal cells in the cochlear aqueduct and scala tympani of the cochlea contained viral antigen. Measles antigen in membranous labyrinth was limited to cells in the organ of Corti and crista of the semi-circular canals (Fig. 1c). The cells of the surrounding endolymphatic membrane were not involved. Again neurones of the cochlear and vestibular ganglia stained specifically.

Sections of saline-inoculated control animals showed no specific fluorescence when stained with viral antisera, and sections of all virus-inoculated animals were negative when stained with non-immune antisera.

Histological examination of sections stained with haematoxylin and eosin showed only minimal inflammatory responses elicited by influenza and mumps virus. Measles virus, however, caused giant cell formation in the spiral ganglia and occasionally in the organ of Corti.

These studies have demonstrated the feasibility of infecting inner ear structures in an experimental animal either by direct inoculation into the labyrinth or indirectly from the subarachnoid space through the cochlear aqueduct. Endolymphatic structures are, however, more readily infected using percutaneous intralabyrinthine inoculation. Preservation of the delicate membranous structures of the inner ear as well as preservation of viral antigens has been possible using paraffin embedding following cold alcohol fixation.

These initial studies have shown that cells of the inner ear differ in their vulnerability to viral infections. Each virus caused a different pattern of infection: influenza virus infection was limited to perilymphatic structures, mumps virus to endolymphatic cells, and measles virus involved both perilymphatic cells and the organ of Corti and the crista. All three viruses infected ganglion cells of the eighth cranial nerve. It is significant that both measles and mumps viruses infected endolymphatic structures or cells in the organ of Corti, since an endolymphatic labyrinthitis has been implicated as the original cause of damage in patients with deafness associated with these two virus infections[1-3].

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Effect of electric fields on growth rate of embryonic chick tibiae in vitro

NUMEROUS investigators have reported work on the stimulation of bone growth by electric currents in the microampere range, with varying degrees of success. Such work, along with the various tentative theories of the underlying mechanisms, have been summarised by Bassett[1], and some quantification of the effect has been attempted[2,3]. Hambury et al.[3] concluded that the necessary crudity of the surgical techniques involved, and the overriding effects of the trauma militated too severely against accurate quantification of the electrically-stimulated bone growth, and proposed[4] that in vitro experiments might yield more definitive results. They also concurred with other workers that if electric currents do influence bone growth, then so should electric and magnetic fields, and Norton[5] has already performed work in this area in vivo, using chickens. We report that the gross development of embryonic chick tibiae grown in vitro is affected by a pulsed, transverse electric field, but that no significant changes were observed when a static, non-varying field was used.

Between one and three bones were placed in each of eight Petri dishes, four of the dishes being subjected to a 1,000 V cm⁻¹ electric field, and four being placed between the inactive plates of a second control apparatus (see Table 1 for detailed method). Each bone was measured before and after a 9 d incubation period. The ratio of the final to the initial bone length was calculated for each bone, giving the fractional increase in length in each case. Also, the experiments were systematised so that the two ratios obtained for the bones from each embryo could be related and compared. Where bones distorted somewhat, the lengths of the longitudinal median lines were measured incrementally. Table 1 gives the results in terms of the fractional increase in length

Table 1 Effect of an invariant electric field on embryonic chick tibiae growth in vitro

| Batch | Fractional length increase | | | 'One-tail' t test | | |
	Test	Control	Test/Control	t	d.f.	Confidence limit
A	1.50	1.50	1.00			
	1.42	1.57	0.90			
	1.64	1.92	0.85			
	1.29	1.43	0.90	1.52	6	>5%
	1.67	1.67	1.00			(less growth)
	1.58	1.67	0.86			
B	1.46	1.26	1.16			
	1.27	1.24	1.02			
	1.31	1.21	1.08			
	1.18	1.25	0.94	1.35	7	>10%
	1.34	1.28	1.05			
	1.34	1.25	1.07			
	1.58	1.44	1.04			
	1.43	1.38	1.04			
C	1.46	1.60	0.91			
	1.43	1.48	0.97			
	1.42	1.37	1.04			
	1.43	1.50	0.95	0.68	7	Not significant
	1.57	1.48	1.06			
	1.49	1.44	1.03			
	1.44	1.41	1.02			
	1.30	1.47	0.88			
D	1.59	1.34	1.48			
	1.41	1.48	0.95			
	1.48	1.44	1.03			
	1.43	1.45	0.99	1.67	7	>5%
	1.64	1.21	1.36			
	1.50	1.51	0.99			
	1.39	1.35	1.03			
	1.46	1.46	1.00			
E	1.30	1.16	1.12			
	1.19	1.16	1.03			
	1.19	1.15	1.03			
	1.20	1.19	1.01	0.65	6	Not significant
	1.14	1.09	1.05			
	1.20	1.21	0.99			
	1.14	1.26	0.90			
Overall	1.40	1.39	1.01	0.21	37	Not significant

The 8–9-d-old embryos were aseptically decapitated, and extracted and the tibiae carefully excised. Each bone was then placed on a thin siliconised cellulose acetate mat supported by a 3 mm thick sponge of similar material, both items being chemically clean and sterile. Each assembly, consisting of a sponge, mat and two or three bones was placed in a 35 mm disposable polystyrene Petri dish containing sufficient medium to thoroughly soak the sponge without immersing the bone. The bones must be allowed free exposure to the incubator atmosphere of CO_2-air (5:95). Chemically-defined BGJ-b nutrient medium[6] was used, as described previously. In particular, the medium was changed every 2 d by gently removing the cellulose mat and placing it on a new sponge in a new Petri dish. This enabled most of the metabolic by-products of the preceding 2 d to be removed in the interstices of the sponge, the mat to which the bones adhered being too thin to retain more than a very small portion of these products. The bones were incubated for 9 d at 37 °C, after which they reached their maximum length. This period was defined by trials in which the length of each bone was measured and plotted against time until the graph became horizontally asymptotic. These measurements were made outside the Petri dishes using a micrometer scale mounted on a dissecting microscope eyepiece. After excision, one bone from each embryo was placed in a Petri dish inserted between the plates of the apparatus applying the electrical field. The other bone was placed in a dish within a similar, but inactive, apparatus as control. Both sets were placed in the same incubator. The apparatus for applying the field consisted essentially of two circular stainless steel plates, 12.7 cm in diameter, and held 1.4 cm apart by a Perspex framework. A Keithley model 244 high voltage power supply was used to apply 1,400 V across the plates, so that a uniform invariant field of 1,000 V cm⁻¹ existed between them, the lower plate being grounded. A Plexiglass disk, 3 mm thick, drilled with four holes 38 mm in diameter, was used to slide the four 35 mm Petri dishes into the space between the stainless steel plates. In the fully operating system, an interlock microswitch was incorporated into the incubator so that the opening of the door switched off the power supply. The stainless steel plates of the second apparatus, containing the controls, were short-circuited and grounded.

of each bone subjected to the field, compared with that for each corresponding control bone from the same embryo. The ratio of each pair of fractional increases is also included. Further, a 'one-tail' *t* test was applied to each batch, and also to the complete series. This shows that there is no significant difference between the test and control bone fractional increases.

A second series of experiments was carried out using a 1,000 V cm^{-1} field pulsed at approximately 1 pulse s^{-1} using a high voltage relay driven at this pulse repetition rate by a unijunction transistor circuit. The live (upper) plate was alternately connected to the $-1,400$ V supply and to ground by this means, so that a good square wave of approximately 1 : 10 mark–space ratio was obtained. Table 2 shows that for batch A, the chances that the pulsed electric field had no effect were no more than 0.5%; and for batches C and E, were no more than 2.5%. These results can therefore be considered significant, whereas those for batches B and D could not, being more than 10 and 25%, respectively. The 'one-tail' *t* test was also applied to the entire series taken as a whole (Table 2). The overall confidence limit was less than 0.5%, clearly very significant.

Bones were photographed in their Petri dishes at the end of each test to provide a record of their macroscopic appearance, then fixed in neutral formalin for 3 d. After manual processing, they were embedded in paraffin wax and sectioned longitudinally. The sections were then fixed to

glass slides by means of a glycerin–albumin mix and dewaxed in a hot air oven. They were then regressively stained with haemotoxylin, counterstained with eosin, and examined with an optical microscope. This did not reveal any differences between test and control slides; nor did a macroscopic inspection of the photographs reveal any differences in growth patterns.

We propose to grow further batches and perform more comprehensive tests including cell counts, wet and dry weight measurements and DNA content analyses, and to determine the effect of variation in field strength, frequency, waveform, and the spatial relationship of the field to the bone.

If our trials are significant, it can be concluded that there is present in the embryonic bone a transducer mechanism which allows the electric field to interact directly and modify (at least) the growth rate. Since the bones are in a pre-osseous phase, this mechanism clearly cannot be related to any properties of hydroxyapatite, thus ruling out at least one mechanism, the collagen–hydroxyapatite semiconductor interface[1]. In addition, because only pulsed electric fields (as opposed to invariant fields) have an effect, it is likely that repeated cycles of charge separation are involved. This is equivalent to applying very small pulsed currents, and since such currents can also be induced by pulsed magnetic fields, a series of experiments using such fields has been initiated. Note that Bassett *et al.* have reported that pulsed magnetic fields do modify bone growth patterns *in vivo*[7].

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Table 2 Effect of a pulsed electric field on embryonic chick tibiae growth *in vitro*

Batch	Test	Control	Test/control	*t*	d.f.	Confidence limit
A	2.56	1.64	1.56			
	2.70	2.10	1.29			
	2.36	1.86	1.27			
	2.62	2.21	1.24	4.49	6	<0.5%
	2.32	2.25	1.03			
	2.40	2.17	1.11			
	2.50	1.87	1.34			
B	2.66	2.25	1.18			
	2.40	2.14	1.12			
	2.24	2.15	1.04			
	2.15	2.00	1.07	1.24	7	>10%
	2.00	1.91	1.05			
	2.30	2.43	0.95			
	2.43	2.09	1.16			
	2.05	2.24	0.92			
C	1.80	1.45	1.24			
	2.11	1.50	1.41			
	1.68	1.65	1.02			
	1.62	1.43	1.13	3.32	7	<2.5%
	2.06	1.37	1.50			
	1.83	1.62	1.13			
	1.74	1.65	1.05			
	1.83	1.78	1.03			
D	1.67	1.52	1.10			
	1.45	1.50	0.97			
	1.74	1.67	1.04			
	1.67	1.74	0.96			
	1.32	1.57	0.84	0.58	9	>25%
	1.39	1.61	0.86			
	1.56	1.05	1.49			
	1.58	1.72	0.92			
	1.67	1.25	1.34			
	1.64	1.60	1.02			
E	2.00	1.96	1.02			
	2.04	1.92	1.06			
	2.03	1.74	1.17			
	2.36	1.68	1.40	2.80	7	<2.5%
	2.00	2.03	0.99			
	1.90	1.87	1.02			
	2.22	1.82	1.22			
	1.90	1.76	1.08			
Overall	2.01	1.80	1.12	2.81	40	<0.5%

Note: column header for "Test/control" appears as "Test / control" (Fractional length increase columns: Test, Control, Test/control; 'One-tail' *t* test columns: *t*, d.f., Confidence limit).

Left-handed to right-handed helix conversion in *Salmonella* flagella

ALTHOUGH bacterial flagella can assume various helical configurations[1-3], the 'normal' and 'curly' configurations are encountered most frequently in nature. In *Salmonella*, normal and curly flagella have helical pitches of about 2.3 and 1.1 μm, respectively[4]. Macnab and Koshland[5] observed that normal flagella of living *Salmonella* were left-handed helical filaments, as shown for *Proteus* and *Bacillus*[6]. Flagella with this handedness and rotating counterclockwise (looking in the direction of travel), would cause helical waves to propagate distally and provide forward thrust[7-9]. Normal flagella can transform reversibly into the curly type when physiological conditions, such as *p*H, are varied[10,11]: this has been termed biplicity. Asakura *et al.*[12], using *Salmonella* strain SJ670 and others, found that reconstituted flagellar filaments also could be transformed reversibly, although they could not control this transformation completely. SJ670 is a motile strain that produces
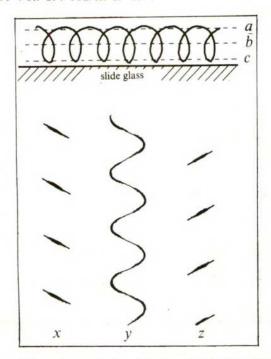
Fig. 1 The optical method used for the determination of helical handedness. For explanation, see text.

the difficulty of photographing individual filaments, assuming that the helical form of individual filaments changed little with association. The optical system consisted of an Ushio 100 W mercury arc lamp, and a Nikon SKR microscope equipped with an Olympus d.c. condenser (numerical aperture 1.2–1.33), an Olympus Apo 40 × objective (numerical aperture 1.0), Nikon 20 × eyepiece and a Nikon EFM camera. In this system, there are no beam reflections and therefore micrographs are real, not mirror images.

The method used for the determination of helical handedness can be understood by considering a bundle of left-handed helical filaments, lying on the surface of a slide glass (Fig. 1). If parts of the bundle near planes a, b and c are illuminated successively and focused by raising the specimen stage discontinuously, then images of types x, y and z will be obtained in succession. If a bundle of right-handed helical filaments is examined, images of types z, y and x will be observed, corresponding to planes a, b and c, respectively.
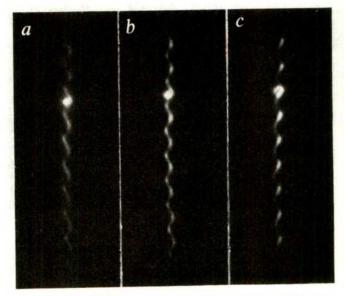
Fig. 2 A set of micrographs obtained for a bundle of normal-type filaments (SJ670) at pH 7.0. Micrographs (a), (b) and (c) were obtained by successively raising the specimen stage. The scale represents 5 μm.

normal flagella. On the other hand, the mutant SJ30 isolated by Iino[13] produces flagella which are stable in the curly configuration in various conditions. The mutation is in the structural gene for the flagellar protein subunit, flagellin, and the mutant cannot swim normally but appears to tumble continuously. Now, using SJ670 and SJ30, we have found that, whereas the normal configuration of flagella is a left-handed helix, the curly configuration is a right-handed helix.

Flagellins were purified from the two strains and polymerised into flagellar filaments as before[12]. Flagellins of SJ670 and SJ30 repolymerised into normal and curly filaments, respectively, which were used in the experiments. We also used curly filaments obtained by copolymerising flagellins of SJ670 and SJ30 at a protein ratio of 9:1[14]. Other curly filaments used were composed of SJ670 flagellin at pH 4.2, for we have found that such filaments are transformed reversibly when the pH changes from 7.0 to 4.2. (Full details will be reported elsewhere.)

If flagellar filaments are suspended in a medium containing methylcellulose (about 0.5% w/v), they associate into bundles visible by dark-field light microscopy, and heterogeneous in thickness (as judged by brightness) and overall length. They are helix-shaped, however, with a pitch equal to that reported by Pijper et al.[4] (Table 1). We have determined the helical handedness of bundles, because of

Figure 2 shows a set of micrographs obtained for a bundle of normal filaments. Clearly the bundle had a left-handed helical form. From this and many other observations we confirmed that normal flagella are left-handed helical filaments. In contrast, curly filaments of each kind examined were found to have a right-handed helical form (Fig. 3). Previously normal and curly configurations of flagella were believed to have the same helical handedness[3,14]. It is important that helical handedness is reversed when filaments transform from normal to curly when the pH changes, for this means that the same protein can form filaments with left or right-handed helical symmetry.

Others have argued that in a genus such as Salmonella and Escherichia, in which the cells are peritrichously flagellated, each organism swims by rotation of the left-handed helical flagella in a counterclockwise sense and tumbles by reversal of the sense of flagellar rotation[7-9]. This argument is based on the observations of the manner in which tethered bacteria swim. We observed that bacteria of SJ30 tethered on to the surface of a slide glass also spin counterclockwise. Therefore, it is likely that in SJ30, the right-handed helical flagella are rotating counterclockwise, giving rise to reverse thrust and continuous tumbling. At a recent meeting (First International Congress of the International Association of Microbiological Societies, Tokyo, September

Table 1		Four specimens examined			
Flagella	pH	Concentration of added NaCl	Helical pitch* (μm)	Type	Helical handedness
SJ670	7.0	0.01 M	2.35	Normal	Left
SJ670	4.2	0.3 M	1.13	Curly	Right
SJ30	7.0	0.01 M	0.99	Curly	Right
9:1 Copolymer of SJ670 and SJ30	7.0	0.01 M	1.04	Curly	Right

Each sample solution was prepared as follows. To a flagella solution containing 0.1 mg ml⁻¹ protein, 0.01 or 0.3 M NaCl, 5 mM citrate and 10 mM Na$_2$HPO$_4$ was added 2 N NaOH or HCl to give pH 7.0 or 4.2, and then the solution was mixed with an equal volume of a solution containing 1% methylcellulose and 0.01 or 0.3 M NaCl.
* Experimental errors were less than 5%.

Fig. 3 Two sets of micrographs obtained for bundles of curly-type filaments: SJ670 (wild type) at pH 4.2 (top) and SJ30 curly mutant at pH 7.0 (bottom). In each set, micrographs (*a*), (*b*) and (*c*) were obtained by successively raising the specimen stage. The scale represents 5 µm.

1974), R. M. Nacnab reported that during bacterial tumbling induced by high intensity blue light[5], or during spontaneous tumbling in uncoordinated mutants, normal filaments were transformed to the curly configuration. Also, in spite of the findings that reversal of rotation occurs during tumbling[7-9], wave propagation was always observed to proceed distally. From these observations he predicted that transformation from normal to curly by hydrodynamic forces was accompanied by a left-handed to right-handed helix conversion, in agreement with our observations. Macnab's finding suggests that the conversion from normal to curly (left-handed to right-handed) configuration of flagella plays a role in the mechanism of tumbling and therefore tactic responses.

It is well known that when peritrichously flagellated bacteria swim in a medium containing methylcellulose, the flagella attached to each cell associate into bundles each with the appearance of a rotating helix. We have found that reconstituted flagellar filaments also associate into bundles in the presence of methylcellulose, though the nature of inter-filament interaction is not well understood. Asakura and Oosawa[15] pointed out that if a macromolecular substance (in this case, methylcellulose) is added to a suspension of large particles (flagellar filaments), an attractive force appears between particles, even when there is no specific interaction between macromolecule and particle. This force is of entropic origin, since the volume which the added macromolecules can occupy is increased to some extent if the particles aggregate. This kind of force may be involved in the inter-filament interaction.

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Prevention of induced leukaemia in mice by immunological inhibition of adenohypophysis

THE theoretical and technical approach to an immunological inhibition of the adenohypophysis (AH) has been discussed in previous work[1,2]. It has been shown that heterologous antibodies against mouse or rat AH have high organ-specificity and affinity for acidophilic cells of the anterior pituitary gland producing growth hormone. The possible application of such an immune intervention in the prophylaxis and therapy of hormone-dependent or hormone-sensitive tumours has been suggested[1] and experiments have demonstrated that hormone-dependent mammary carcinoma in rats can be prevented by anti-AH serum treatment[3].

Further, the thymus is primarily involved in experimental leukaemogenesis in mice and thymectomy prevents or delays the onset of leukaemia induced by X rays or chemical carcinogen[4,5]. In view of the extraordinary sensitivity of the thymus to hormonal change in general and especially to that of growth hormone[6], we suggested that inhibition of cells of the AH secreting growth hormone (or prolactin) might effect lymphoid cell turnover in the thymus and therefore influence the onset of leukaemia[1]. Recently, endocrine derangements have been found in SJL/J mice, a strain characterised by a very high incidence of spontaneous reticulum cell neoplasms (RCN), and this observation prompted the present investigation on the role of hormone in leukaemogenesis[7]. We have therefore investigated the effect of anti-AH serum in mice treated with different leukaemia-inducing agents.

Three groups of C57BL/6 inbred female mice (50 d old) received four doses of 170 rad wholebody irradiation at weekly intervals. At 34 d after the first irradiation, group 1 was injected intraperitoneally with 0.5 ml of sterile sheep anti-C57BL/6 AH serum, prepared and titrated as described previously[1,8]. Group 2 was inoculated with the same amount of normal sheep serum (NSS) and group 3 was untreated. The same quantities of anti-AH serum or NSS were sub-

Table 1 Effect of anti-AH serum on lymphosarcoma induction by X rays in C57BL/6 mice

Host treatment	Lymphosarcoma incidence	Average latent period (d)
Anti-AH serum	6/19 (31%)	245
NSS	19/22 (86%)	201
—	11/14 (78%)	198

sequently inoculated 48, 66, 96, 175 and 210 d after the first irradiation.

Using previously described techniques[9], three groups of SJL/J female mice (50 d old) were administered by stomach tube five doses of the carcinogen 7,12-dimethylbenzan-thracene (DMBA) at weekly intervals. A dose of 0.1 ml of the DMBA solution (1 mg DMBA) in polyethylene glycol 400 was given at each feeding. At 31 d after the first feeding, one group was injected intraperitoneally with 0.5 ml anti-AH serum, one group was injected with the same quantity of NSS and one group was untreated. At 34, 48, 71, 93, 118, and 142 d after the first DMBA feeding, further doses of 0.5 ml anti-AH serum or NSS were injected. Because of the danger of anaphylactic shock in SJL/J mice, which are exceedingly susceptible to sensitisation with heterologous proteins, increasing doses (0.2; 0.4 ml of serum diluted 1:10; 0.1 ml undiluted serum) were

Table 2 Effect of anti-AH serum on lymphosarcoma induction by DMBA in SJL/J mice

| Host treatment | Lymphosarcoma | | Reticulum cell neoplasms | | Other tumours |
	Incidence	Average latent period (d)	Incidence	Average latent period (d)	
Anti-AH serum	12/39 (31%)	227	8/39 (20%)	299	2/39 (5%)
NSS	27/35 (77%)	134	2/35 (5%)	320	1/35 (2%)
—	9/15 (60%)	161	2/15 (13%)	253	3/15 (20%)

given respectively 3, 2 and 1 d before the final dose of 0.5 ml anti-AH serum or NSS.

Both treatments of either X rays in C57BL/6 mice[10] or DMBA in SJL/J mice[9] induce high incidences of lymphosarcomas. Both X rays and DMBA had no effect on the development of spontaneous RCN. Tables 1 and 2 show that inoculation with anti-AH antibodies during and after treatment with carcinogenic agents drastically reduced the incidence of lymphosarcoma in both experimental models. Further, the antiserum prolonged the average latent period for the development of the disease. On the other hand, treatment with anti-AH serum (Table 2) did not influence development of RCN in SJL/J mice.

Many direct and indirect data support the hypothesis that host factors of endocrine character are of primary relevance for development of systemic neoplasia[7,11]. Our data support this contention by demonstrating that immunological inhibition of the adenohypophysis influences both the incidence and time of onset of lymposarcoma in mice.

The mean latent period for spontaneous RCN is beyond 400 d and that for lymphatic leukaemia in DMBA-treated SJL/J mice is 130–170 d. The low incidence of RCN in the experiment with SJL/J mice (Table 2) probably results from the fact that the experiment was terminated after one year. Anti-AH serum does not influence incidence and does not delay onset of spontaneous RCN in SJL/J mice (W. P. and N. H-G., unpublished), thus pointing out the remarkable selectivity of action of anti-AH serum for lymphosarcoma. Both organ-specificity and potency of the anti-AH antibodies can undoubtedly be enhanced by further studies. It is therefore most astonishing that such a striking reduction in incidence of leukaemia was obtained in both experimental models in view of the relatively empirical dosage of the antibodies. It will be of particular interest to determine whether this distinctive kind of immune intervention will be similarly effective in preventing naturally occurring leukaemia, such as that in AKR mice. These data encourage new approaches to therapy of lymphatic leukaemia by 'immunological hypophysectomy'.

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Oxygen dissociation properties of human embryonic red cells

THERE are three stages in the development of human haemoglobins. In the smallest embryos studied the embryonic haemoglobins, Hb-Gower 1, Hb-Gower 2 and Hb-Portland are the main haemoblobins found[1], but foetal haemoglobin (Hb-F) is also present in gradually increasing amounts. By about the 30 mm (crown–rump (CR) length) stage, Hb-F amounts to about 50% of total haemoglobin and after 50 mm it forms over 90% of total pigment. Adult haemoglobin (Hb-A) is already present by week 8 of gestation but this does not exceed 10% of total haemoglobin until week 30; after this it gradually increases until at term it forms between 10% and 30%. After birth, Hb-F is rapidly replaced by Hb-A and at 6 months Hb-F is less than 10%, while at one year it forms less than 2% of total haemoglobins. The oxygen dissociation properties of adult haemoglobin and of foetal haemoglobin have been studied in great detail. Foetal blood has a higher oxygen affinity than adult blood; this helps the transport of oxygen across the placenta and ensures that the steep part of the oxygen dissociation curve corresponds to foetal tissue oxygen tensions, thus maximising the release of oxygen. The oxygen affinities of the human embryonic haemoglobins have so far not been studied because the amounts available have been extremely small. Here we describe the oxygen dissociation properties of human embryonic red cells which were found to be similar to those of foetal red cells.

Cord blood samples were taken from embryos and foetuses obtained from terminations of pregnancy carried out for unrelated maternal indications. Great care was taken to avoid contamination by maternal blood and the electrophoretic patterns obtained confirmed that this was not a problem. The red cells were washed three times in isotonic NaCl and the oxygen dissociation curves of the red cells suspended in isotonic phosphate buffers pH 7.13 (or pH 7.46) at 37 °C were measured[2]. In the method used the saturation of the haemoglobin with oxygen is measured spectrophotometrically using the second sample position of a Unicam SP 1800 or SP 800(0) recording spectrophotometer. Because of the small amount of cells the absorbance scale expansion was used. First the haemoglobin was deoxygenated using a vacuum and the spectrum recorded, then a measured amount of air was let into the tonometer and after equilibration at 37 °C the spectrum was again recorded. This procedure was repeated until the haemoglobin was fully saturated with oxygen. The partial pressure of oxygen P_{O_2} in the tonometer and the percentage saturation of haemoglobin

Block 1

Position in molecule	E	E	E	I	E———E	I	E	E	E	E	E	E
Residue No. (N Terminus)	7	8	9	10	11———16	17	18	19	20	21	22	23
Postulated sequence of ζ-chain	(LYS)	THR	SER	LEU	LYS ———(LYS)	ILE	THR	SER	ASN	ALA	THR	GLU
Amino acids found in known α-chains	LYS	THR LYS (ala) (gly) (asn)	ASN (his) (ala)	VAL ILE	LYS ——— LYS	VAL ILE	GLY ALA (pro)	GLY ALA (pro) (ser)	HIS ASX (lys)	ALA	GLY ALA (glu) (asp)	GLU gly (asp)

Block 2

	I	I	E	E	I	I	$\alpha_1\beta_1$ contact	$\alpha_1\beta_1$ contact	I haem	I	E $\alpha_1\beta_1$ contact	I $\alpha_1\beta_1$ contact	I $\alpha_1\beta_1$ contact
	24	25	26	27	28	29	30	31	32	33	34	35	36
	ILE	GLY	THR	GLU	THR	LEU	GLU	ARG	LEU	LEU	HIS	SER	PHE
	TYR (ala) (ile)	GLY (val)	ALA (gly) (ala) (thr)	GLU	ALA (gly) (thr)	LEU (val)	GLU (asp) (gly)	ARG	MET (thr)	PHE (leu)	LEU (ala) (gln) (his) (thr) (ile)	SER (gly) (val)	PHE (tyr)

Block 3

E $\alpha_1\beta_2$ contact	E $\alpha_1\beta_2$ contact	Haem	E deoxy $\alpha_1\beta_2$ contact	E	E	Haem 'Distal His'	I	E	E	E	E
37	38	39	40——56	57	58	59	60	61———89	90
PRO	GLN	THR	LYS———(LYS)	ALA	HIS	GLY	SER	LYS———(LYS)	ILE
PRO	THR (gln)	THR	LYS ——— LYS (gln)	GLY (ala)	HIS	GLY	LYS (gln)	LYS ——— HIS (tyr) (ser)	LYS

Block 4

E	E———CC	SC CC	I Haem	CC	CC $\alpha_1\beta_1$	I $\alpha_1\beta_1$	I	I $\alpha_1\beta_1$	I $\alpha_1\beta_1$
91	92———99	100	101	102	103	104	105	106	107
LEU	ARG———(LYS)	ALA	LEU	SER	HIS	CYS	LEU	GLY	LYS
LEU	ARG——— LYS	ALA (ile)	LEU	SER (gly) (ala)	HIS (glu) (asn)	CYS (ser) (his)	LEU (phe) (ile)	LEU (val)	VAL (ser)

Block 5

I	I	I	SC $\alpha_1\beta_1$	E	SC	E———$\alpha_1\beta_1$	CC	I	I	CC
108	109	110	111	112	113	114———127	128	129	130
GLN	LEU	ALA	SER	HIS	LEU	TYR (chymotryptic split)———(LYS)	PHE	LEU	ALA
THR (val) (gly)	LEU (phe) (ile)	ALA (val) (met)	CYS ALA (val) (ser) (asn) (phe)	HIS (tyr)	LEU (his) (val)	PRO——— LYS	PHE	LEU (phe)	ALA (ser) (thr) (cys) (gln)

Block 6

CC	I	CC	CC	SC CC	I	CC	CC	E	$\alpha_1\beta_2$	E
131	132	133	134	135	136	137	138	139	140	141
SER	VAL	SER	GLN	VAL	LEU	THR	SER	LYS	TYR	ARG
SER ASN (ala)	VAL (leu)	SER (gly) (ala)	THR (leu)	VAL (ala)	LEU	THR (ser) (asx)	SER ala glu	LYS	TYR	ARG

Fig. 1 In this figure the amino acid composition of peptides from the ζ-chain determined by Capp *et al.*[8] are arranged to give a postulated sequence corresponding to that of the known α chains[9]. It can be seen that the fit is very good except at positions 90 and 114 which are external and position 107 in the $\alpha_1\beta_1$ contact. E, External; I, Internal; haem, haem contact; (LYS) in brackets in the postulated sequence assumed to be present from tryptic cleavages seen. Amino acids found in known α chains: Caps—common, lower case and brackets—rare. ↓, Tryptic cleavages. The positions of the various residues in the haemoglobin molecule are from the data of Perutz and his coworkers (for refs see ref. 7).

with oxygen were then calculated[3]. The use of the second sample position of the Unicam SP 1800 (SP 800(0)) recording spectrophotometers allows the direct recording of spectra of intracellular haemoglobin with minimal loss of precision as a result of light scattering.

Oxygen dissociation curves were obtained from the red cells from six embryos with a CR length of less than 35 mm. In these cells the embryonic haemoglobins amounted to between 30 and 50% of total haemoglobin. Similar studies were carried out on red cells obtained from several foetuses (Table 1). The oxygen affinities of the embryonic red cells do not differ from those of foetal red cells in the conditions used, the P50 (partial pressure of oxygen at 50% saturation of haemoglobin with oxygen), Bohr shift and haem–haem interactions being very similar. In order to exclude the presence of high or low affinity components the partial pressures of oxygen necessary for 20 and 80% saturation were also compared and found to be identical. These studies therefore show no difference in the oxygen dissociation properties of embryonic red cells compared with foetal red cells and further studies are necessary to elucidate the physiological significance of these haemoglobins.

Table 1 Comparison of the oxygen affinity of human embryonic* and foetal red cells suspended in isotonic phosphate buffers

Red cells suspended in isotonic buffer pH 7.13	Embryonic red cells (6 samples)	Foetal red cells
p20 mmHg	13–17	12–16
p50 mmHg	25–30	22–27
p80 mmHg	38–46	38–46
Haem–haem interactions (n value†)	about 2.5	about 2.6 normal adult red cells
In isotonic buffer pH 7.46	(4 samples)	
p20 mmHg	7.5–9.5	9–10.5
p50 mmHg	18–20	17.5–18
p80 mmHg	29–32	27.5–28
Bohr shift	−0.43 to −0.49	−0.42 to −0.52

*Cells from embryos with a crown–rump measurement of less than 40 mm and containing more than 30% embryonic haemoglobins.
†The slope of the line log $y/(1-y)$ against log P_{O_2} where y is the fractional saturation with oxygen as a measure of the haem–haem interactions.

As haem–haem interactions are thought to require a pair of different chains (such as $\alpha_2\beta_2$) in each haemoglobin molecule and do not occur in haemoglobins consisting of one type of chain only (such as Hb-β_4 or Hb-γ_4), our finding that the haem–haem interactions of embryonic haemoglobins were normal argues against the presence of a major haemoglobin species containing one type of chain only, as previously suggested for Hb-Gower 1 (ref. 4). Studies of the embryonic haemoglobins have shown that there are two types of embryonic globin chains, ε-chains and ζ-chains. The ε-chain is found in Hb-Gower 2 in combination with α-chains, Hb-$\alpha_2\epsilon_2$. The other embryonic chain, the ζ-chain, is found in combination with γ-chains in Hb-Portland, $\zeta_2\gamma_2$ (ref. 5), and in combination with β-chains, Hb-$\zeta_2\beta_2$ (ref. 6), and it has been suggested that the ζ-chain is an α-type chain[7]. Comparison of the composition of some tryptic peptides from the ζ-chain with that of other mammalian haemoglobin α-chains (Fig. 1) confirms this idea. Previously it has been shown[4] that Hb-Gower 1 contains ε-chains but not any α^A-chains. Our observation that the haem–haem interactions are the same in embryonic red cells as in foetal or adult red cells indicates that Hb-Gower 1, which forms a large haemoglobin component in the former, must contain α-like chains as well as ε-chains. It is, therefore, suggested that it consists of ζ-chains combined with ε-chains: Hb-$\zeta_2\epsilon_2$.

Note added in proof: The peptide described by Capp *et al.*[8] from their ζ-chain preparation from Hb-Portland which could not be fitted to the α-chain sequence (Fig. 1) appears to correspond to the first part of the additional peptide found[10] attached to the

C-terminal end of the α-chain in Hb-Icaria present in some cases of α-thalassaemia.

Residue no.	142	143	144	145
Hb Icaria	LYS	ALA	GLY	ALA
Postulated additional peptide from ζ-chain	LYS	ALA	GLY	ALA
Residue no.	146	147	148	149
Hb Icaria	SER	VAL	ALA	VAL
Postulated additional peptide from ζ-chain	SER	LEU	THR	THR

It is not clear however whether this peptide is present in all samples studied (that is, those from α-thalassaemia as well as those from normal embryos).

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Progesterone antagonism of the oestrogen receptor and oestrogen-induced uterine growth

PROGESTERONE has long been considered an antagonist of oestrogen action[1]. The delicate balance and interactions between these ovarian hormones are essential for many reproductive functions. Early studies in chick oviducts and uteri of rats and mice have shown that the simultaneous administration of progesterone and oestrogen resulted in inhibition or modification of oestrogen-induced growth of these target organs[2-5]. One possible mechanism by which progesterone could be antagonistic to oestrogen is by suppressing the quantity of cytoplasmic oestrogen receptor. It is generally held that the mechanism by which oestrogen, O, stimulates uterine growth depends on the binding of O to cytoplasmic receptors, R_c, to form R_cO complexes[6]. These R_cO complexes are translocated to nuclear sites where they probably stimulate nuclear events that cause the uterus to grow[7]. Translocation and nuclear accumulation of receptor–oestrogen complexes, R_nO, are accompanied by a concomitant decrease in the quantity of R_c (ref. 8). During the period when R_c is reduced, the uterus is insensitive to additional exogenous oestrogen[9]. Gradually R_c is replenished by processes which may involve reutilisation and/or resynthesis[8]. Work from our laboratory indicates that the mechanism of action of non-steroidal oestrogen antagonists resides in their inability to stimulate the replenishment of the cytoplasmic oestrogen receptor, R_c, thereby rendering oestrogen responsive tissues less sensitive to oestrogen[10,11]. The possibility that progesterone might also act as an oestrogen antagonist by reducing the amount of oestrogen R_c has been suggested[12,13]. In this report, we demonstrate that progesterone does decrease the quantity of oestrogen receptors by interfering with the replenishment of R_c and that this decrease results in a reduced sensitivity of uterine tissue to oestrogen.

To establish the effect of progesterone on the quantity of oestrogen receptor, it is necessary to use assay techniques that permit the measurement and distribution of total receptor[14,15]. The ^{3}H-oestradiol exchange assay, as developed in this laboratory and elsewhere[15,16] enables us to make such measurements.

Table 1 Effect of progesterone on various uterine growth parameters and concentration of cytoplasmic oestrogen receptor

Pretreatment	Uterus (day 4)			Cytoplasmic receptor (day 4)	
	Wet weight (mg ± s.e.m.)	Protein (mg ± s.e.m.)	DNA (μg ± s.e.m.)	pmol per 100 mg protein	pmol per mgDNA
a (OL)	92.6 ± 4.5	8.81 ± 0.41	679.7 ± 17.8	27.1 ± 2.3†	3.52 ± 0.31†
b (OL + P)	69.0 ± 2.3†	8.20 ± 0.57*	744.5 ± 26.1†	13.2 ± 4.9†	1.47 ± 0.05†

Immature rats were treated with hormones as described in Fig. 1 and uterine wet weight, protein and DNA content were determined on day 4 by the methods of Lowry *et al.*[24] and Ceriotti[25]. The values represent the mean ± s.e.m. from six to eight experiments with six animals per experiment.
*No significant differences between a and b
†Significant differences ($P < 0.01$) between a and b

Immature female Sprague–Dawley rats (22 d old) were purchased from Texas Inbred Mice Co. and housed in a controlled environment with 14 h of artificial light between 0700 and 2100. All rats received two daily subcutaneous injections of 2.5 μg oestradiol 17-β in 0.5 ml of saline containing 1% ethanol. The pretreatment with oestradiol was done to increase the sensitivity of the uterus for progesterone, presumably through the effect of oestrogen on increased synthesis of the progesterone receptor[17,18], and to maximise the translocation of
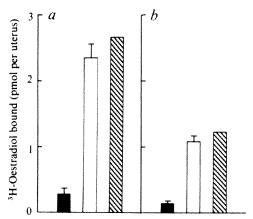
Fig. 1 The effect of progesterone on the quantity of oestrogen receptor in the rat uterus. Two groups of immature rats were given 2.5 μg of oestradiol for 2 d as described in the text. Group a received another injection of oestradiol on day 3 while group b received oestradiol plus progesterone. On day 4 the quantity of oestrogen receptor was determined in the nuclear (■) and cytoplasmic (□) fractions as described in text. The total (///) amount of receptor (■ + □) is also shown. The data represent the mean ± s.e.m. from six to eight experiments with 6 animals per experiment.

the R_cO complex to the nucleus. On the third day rats were divided randomly into two groups. Animals in group A were injected with 2.5 μg of oestradiol in 0.5 ml of saline and 0.2 ml of sesame oil. Animals in group B were injected with 2.5 μg of oestradiol plus 2.5 mg of progesterone dissolved in 0.2 ml of sesame oil. In both groups, the sesame oil or progesterone in oil was injected at a separate subcutaneous site and did not affect the adsorption of oestradiol. On day 4 uteri were removed and analysed for cytoplasmic and nuclear receptor by the ³H-oestradiol exchange assay[15,16].

Progesterone treatment caused a significant reduction in the

amount of cytoplasmic oestrogen receptor in the uterus on day 4 (24 h after the injection, Fig. 1 and Table 1). A reduction was also observed in the amount of R_nO present in the nuclear fraction although these quantities in either group are small and hence have little influence on the total amount of receptor (Fig. 1). Antagonistic effects of progesterone are also observed at this time by the reduced wet weight of the uterus (Table 1). But, the decrease in the quantity of R_c does not result from a general depressive action of progesterone on uterine protein synthesis, since the decrease can still be observed when the data are expressed as sites per mg protein (Table 1).

Since it has been shown that progesterone does not interfere with the ability of oestrogen to cause the translocation of the RO complex from the cytoplasm to the nucleus[15], the reduced R_c 24 h after progesterone treatment probably results from the blockade of the replenishment process. To test this possibility the quantity of R_c was determined at various times after treatment with oestrogen (O), or oestrogen plus progesterone (O + P) (Fig. 2). These data show that the level of R_c is depleted immediately after injection of oestrogen plus progesterone and that replenishment occurs during the next 24 h. In the case of oestrogen plus progesterone, the level of R_c does not exceed the quantity present 24 h earlier. In contrast, oestrogen alone results in a significant increase above control level. This antagonistic influence of progesterone is dose-dependent and specific for progestational hormones (data to be reported elsewhere).

The reduction in the quantity of cytoplasmic oestrogen receptor following progesterone treatment should make the uterus less responsive to oestradiol. To assess this possibility animals were treated with oestradiol or oestradiol plus progesterone as described in Fig. 1 and on day 4 all animals were injected with 2.5 μg of oestradiol. The wet weight, dry weight and protein content of the uterus were examined 24 h later. Oestrogen treatment increased wet and dry weight in both OL and OL + P pretreatment groups when compared with saline injected controls (Table 2). But the ability of oestradiol (OL) to increase uterine weight was significantly depressed ($P < 0.01$) in the OL + P pretreatment group when compared with OL pretreatment group (Table 2). This was also true for the protein content of the uterus (OL pretreatment group, 10.7 ± 0.2 mg per uterus; OL + P pretreatment group, 8.7 ± 0.3 mg per uterus). No appreciable difference among treatment groups was observed, however, for saline-injected controls. These data indicate that the antagonistic effect of progesterone is correlated with its depressive effect on the quantity of cytoplasmic

Table 2 Antagonism of oestrogen uterine growth by progesterone

Pretreatment (day 3)	Treatment (day 4)	Uterine weight			
		Wet weight (mg ± s.e.m.)	% Saline control	Dry weight (mg ± s.e.m.)	% Saline control
a (OL)	OL	151.0 ± 20.0*	228	23.0 ± 1.3*	195
	S	66.1 ± 2.9†	100	11.8 ± 0.5†	100
b (OL + P)	OL	89.0 ± 4.0*	155	17.6 ± 0.6*	166
	S	57.3 ± 2.3†	100	10.6 ± 0.4†	100

Immature rats received hormone treatment as described in Fig. 1. On day 4 they were injected with 2.5 μg of oestradiol (OL) or saline (S) and the uterine weight was determined on day 5. The values represent the mean ± s.e.m. of five experiments with six rats per experiment.
*Significant differences between pretreatment groups ($P < 0.01$).
†No significant differences between pretreatment groups.

oestrogen receptor. When considered in the light of the results from Figs 1 and 2, these data suggest that progesterone decreases cytoplasmic R_c and thereby reduces the sensitivity of the uterus to subsequent oestrogen action.

The antagonistic effects of progesterone on uterine weight and protein content probably reflect the ability of progesterone to redirect the manner in which certain uterine cell types will respond to oestrogen. Histological examination of the uterus on day 5 indicates considerable changes in the epithelial and stromal elements of the uterus (data not shown). In the OL + P pretreatment group, the height of the luminal epithelium is greatly reduced and the surface area of the lumen is expanded. In addition, the quantity of stroma seems to be reduced although the quantity of myometrium is only slightly affected. Thus, progesterone may reduce the amount of R_c in certain cell types and not in others, thereby differentially controlling the action of oestrogen.

Several reports have suggested that progesterone has a depressive effect on the cytoplasmic oestrogen receptor. The cytoplasmic fraction of human uterus binds more oestrogen during the follicular phase than during the luteal phase of the menstrual cycle[19,20]. The ability of the rabbit uterus to bind oestrogen has also been shown to decrease during pseudopregnancy and
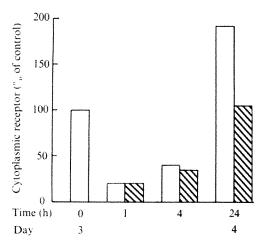
Fig. 2 The effect of progesterone on the oestrogen-induced depletion and replenishment of the cytoplasmic oestrogen receptor. Immature rats received 2.5 μg of oestradiol for 2 d and on the third day they were injected with either oestradiol (□) or plus oestradiol and progesterone (////). The quantity of cytoplasmic oestrogen receptor was determined at 1, 4, and 24 h after injection. The values represent the mean of three experiments with four animals per experiment.

increase subsequent to lutectomy[21]. Thus, in conditions when the concentration of progesterone in the blood is high, the oestrogen binding capacity of the uterus is decreased. Brenner *et al.*[13], made similar observations with the monkey oviduct. In the above studies indirect methods or methods that do not permit the evaluation of the quantity and distribution of total receptor were used. The methods we used permit this evaluation and demonstrate conclusively that progesterone can decrease the quantity of cytoplasmic oestrogen receptor. Also, this decreased quantity of R_c is correlated with a reduced or redirected ability of the uterus to respond to oestrogen.

It is known that oestrogens and progestins bind to separate cytoplasmic receptor molecules[22] and that the receptor-steroid complexes thus formed do not compete for the same nuclear sites[15,23]. Therefore, it seems that progesterone or the progesterone-receptor complex influences the replenishment of the cytoplasmic oestrogen receptor by interfering with its resynthesis or recycling, thereby reducing the quantity of oestrogen R_c in the uterus. This concept, that progesterone can reduce the quantity of oestrogen receptors and thereby reduce or modify

the ability of a tissue to respond to oestrogen, is basic to the elucidation of the progesterone-oestrogen interactions during various reproductive states.

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Immunoperoxidase staining of α-bungarotoxin binding sites in muscle endplates shows distribution of acetylcholine receptors

α-BUNGAROTOXIN (αBT) and similar toxins are specific ligands for the nicotinic acetylcholine (ACh) receptors in skeletal muscle[1,2] fish electric organ[3-5] and retina[6] and other parts of the central nervous system[7,8]. Fluorescent[9,10] and autoradiographic[1,11,12] methods have been used to visualise the αBT binding sites in muscle and electric organ. We report here an αBT-immunoperoxidase staining method for light and electron microscopy, which is relatively convenient and has high resolution. We have used this method to study the distribution of ACh receptors in skeletal muscle.

Mouse diaphragms or frog (*Rana pipiens*) sartorius muscles were agitated gently for 1.5–2 h with 5×10^{-8} M αBT in either F14[13] tissue culture medium with 10% foetal calf serum at 37 °C in a 5% CO_2 atmosphere (mouse), or frog Ringer solution with 2 mg ml^{-1} bovine serum albumin (BSA) at room temperature (frog). Controls were either incubated without αBT or preincubated for 10 min with 10^{-3} M decamethonium (a specific inhibitor of αBT binding) followed by 5×10^{-8} M αBT with 10^{-3} M decamethonium. The tissues were then washed extensively in incubation medium without αBT or decamethonium (the last washes without serum or BSA), fixed for 90 min at 0–4 °C in a solution containing 2% paraformaldehyde, 0.01 M $NaIO_4$, 0.075 M lysine and 0.037 M phosphate buffer, final pH about 6.9 (ref. 14), cut in pieces, washed in 0.1 M sodium phosphate buffer, pH 7.1 (PB), cryoprotected by soaking in 5% dimethylsulphoxide in PB, and quick frozen. For electron microscopy, cryostat sections 20 μm thick were fixed to gelatin-coated plastic dishes with vapours of
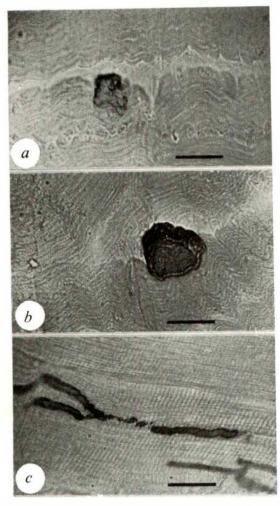
Fig. 1 *a*, αBT-immunoperoxidase-stained endplate in mouse diaphragm. *b*, Acetylcholinesterase-stained endplate in a parallel section. *c*, αBT-immunoperoxidase-stained endplate in frog sartorius muscle. Bright field photomicrographs. Reference bars represent 20 μm.

8% paraformaldehyde at 40 °C, washed with PB, and treated at room temperature as follows: (1) 60 min in rabbit anti-αBT (ABT), 29 μg ml⁻¹, in PB with 0.1 mg ml⁻¹ BSA (PB-BSA); (2) 30-min wash with PB-BSA; (3) 60 min in a horseradish peroxidase conjugate of the IgG fraction from goat antiserum to rabbit IgG (AR-HRP), 13 μg ml⁻¹ in PB-BSA. (ABT was purified from rabbit antiserum to αBT by affinity chromatography on a Sepharose-αBT column. One milligram of ABT precipitated 22 μg of αBT in a precipitin test. AR-HRP was made from the IgG fraction of goat antiserum to rabbit IgG (Miles) by conjugation with horseradish peroxidase (RZ-3.2, Worthington) according to the two-step method of Avrameas and Ternynck[15], except that the conjugation step was done at *p*H 9.0. The conjugate had an A_{403}/A_{280} of 0.49, thus approximately a 1.2 molar ratio of peroxidase to IgG.) Antibody-treated sections were washed extensively with PB-BSA and PB, refixed in 2% glutaraldehyde in PB, washed, and stained for 30 min at room temperature with 3,3′-diaminobenzidine (DAB, 0.1 mg ml⁻¹) and 0.001% H_2O_2 in 0.05 M Tris-HCl, *p*H 7.6 (ref. 16), washed for up to 3 h, postfixed in 1% OsO_4 in PB for 45 min, then dehydrated and embedded in Epon. Except where stated, thin sections were not poststained with uranyl acetate or lead citrate. This omission preserved the high contrast between the DAB reaction product and the tissue background. For light microscopy, cryostat sections 12 μm thick were dried on to glass slides and treated as above, omitting OsO_4 treatment, cleared and mounted under coverslips; alternate sections were stained for acetylcholinesterase[17].

As seen by light microscopy (Fig. 1), the αBT-immunoperoxidase stain was limited to the muscle endplates. The observed patterns of staining resembled the characteristic paths of nerve endings in these species as delineated by silver or gold impregnation. In mouse diaphragm (Fig. 1*a* and *b*) the immunoperoxidase stain gave the endplate the appearance of convoluted strands but the cholinesterase stain was less restricted, filling the endplate region. In frog sartorius, however, both immunoperoxidase (Fig. 1*c*) and acetylcholinesterase stains followed the paths of the nerve endings. Omission of αBT or either antibody from the staining procedure completely eliminated endplate staining. Compounds known to interact specifically with nicotinic ACh receptors were assayed for their effects on αBT-immunoperoxidase staining. Cryostat sections of untreated mouse diaphragm 10 μm thick were incubated for 10 min at room temperature with a 10⁻³ M concentration of one of the following: *d*-tubocurarine, carbamylcholine (with 10⁻⁵ M eserine), nicotine, or decamethonium, in phosphate buffered saline[18] with BSA (2 mg ml⁻¹), and then incubated 30 min more with the addition of 5×10^{-7} or 5×10^{-8} M αBT. The sections were then washed, fixed, and immunoperoxidase stained as described above. All the ligands markedly reduced endplate staining at both concentrations of αBT and all were more effective at the lower concentration. Decamethonium was the most effective blocker, virtually eliminating endplate staining at 5×10^{-8} M αBT concentration. The ability of these ligands to block the staining reaction at the concentrations used is consistent with the known pharmacology of the binding of αBT and similar toxins to ACh receptors[2-5]. These pharmacological results, together with the immunochemical controls mentioned, show the αBT immunoperoxidase stain to be highly specific for nicotinic ACh receptors. Staining of the same specificity was also obtained in diaphragms fixed in paraformaldehyde before freezing, sectioning and incubation with αBT.

Electron microscopy (Fig. 2) confirmed the restriction of staining to the endplate (Fig. 2*a*) and revealed a non-homogeneous distribution of stain within the endplate (Fig. 2*b* and *f*). The heaviest staining was on the muscle plasma membrane at the 'tops' of the postsynaptic folds, with little or no stain at the 'bottoms' of the folds. Some of the stain was on the presynaptic plasma membranes. This was less intense than the postsynaptic stain. A qualitatively similar distribution of stain was seen in the diaphragm of a mouse killed by intravenous injection with 10 μg of αBT. Staining was almost eliminated by the presence of 10⁻³ M decamethonium during αBT incubation (Fig. 2*d*) and completely eliminated by omission of αBT (Fig. 2*c*).

The presence of some presynaptic ACh receptors cannot yet be ruled out by this method; however, it seems that either specific antigen–antibody complex or DAB reaction product originally attached to postsynaptic ACh receptors was translocated and later fixed to the presynaptic membranes. This was most clearly shown by the presence of stain on any structure located directly opposite a site of strong postsynaptic membrane staining. These structures included Schwann cell processes (Fig. 2*b* and *f*), and the muscle basement membranes in the synaptic cleft (Fig. 2*f*) or in denervated muscle (A. N. Bender, S. P. Ringel, B. W. Festoff, W. K. Engel, Z. V. and M. P. D., unpublished), as well as the presynaptic nerve membrane. This spreading of stain could also account for the staining of the membranes in the relatively shallow folds of the frog endplates (Fig. 2*f*).

It is clear that most, if not all, of the muscle ACh receptors are restricted to that region of the postsynaptic plasma membrane which is nearest to the nerve ending and is contiguous with the postsynaptic dense material normally seen in thin sections poststained with uranyl acetate and lead citrate (Fig. 2*e*). This is in agreement with the results of electron microscope autoradiography with radioactive
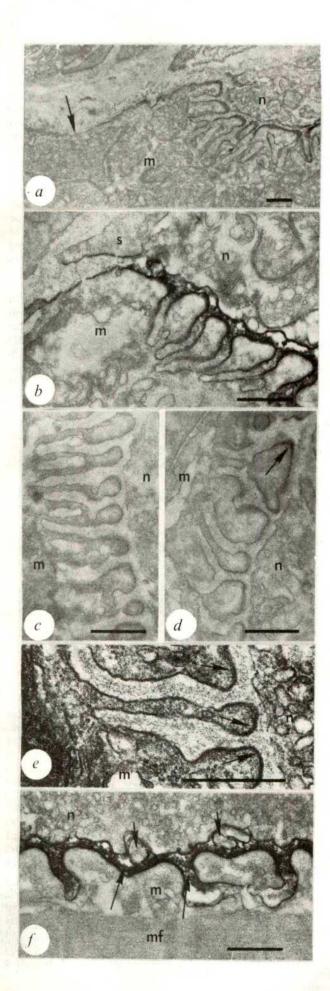
Fig. 2 Mouse diaphragm (*a-e*) and frog sartorius muscle (*f*) endplates stained by the αBT-immunoperoxidase method after various treatments. *a*, αBT-treated. The endplate is stained, but the nearby muscle plasma membrane (arrow) and other structures are not. *b*, αBT-treated. Most of the stain is on the muscle plasma membrane at the tops of the postsynaptic folds. There is some stain in the synaptic cleft and on the nerve and Schwann cell plasma membranes. *c*, Control incubated without αBT. There are no stained structures. *d*, Decamethonium and αBT-treated. There is little or no stain (arrow) on the muscle and nerve plasma membranes. *e*, Control incubated without αBT. This thin section was poststained with uranyl acetate and lead citrate. Note the postsynaptic dense material (arrows). *f*, αBT-treated (frog sartorius). The staining pattern resembles that in mouse. Note the staining of Schwann cell processes (short arrows) and of the basement membrane (long arrows). Reference bars represent 0.5 μm. n, Nerve ending; m, muscle; mf, myofibril; s, Schwann cell process.

αBT[12]. The postsynaptic distribution of ACh receptors revealed by the αBT-immunoperoxidase method also corresponds to that of the 80–120-Å membrane particles observed in freeze-fractured[19-20] and in thin-sectioned[21] muscle.

The αBT-immunoperoxidase method offers high resolution of the ultrastructural distribution of nicotinic ACh receptors and should be useful for light and electron microscopic evaluation of the distribution of ACh receptors in the central nervous system where structural and functional complexity pose serious obstacles to neurophysiological methods of receptor analysis. Our preliminary results with chicken retina indicate that the method will indeed be useful in examining the central nervous system.

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Putative neurotransmitters in clonal cell lines

THE regional distribution of putative neurotransmitters in the mammalian central nervous system (CNS) has been studied often (for example, refs 1–4). Although in some cases neurotransmitter synthesis has been correlated with nervous function, its distribution among different cell types has not been assayed. For example, the distribution of the putative neurotransmitters taurine and γ-aminobutyric acid (GABA) between nerve and glia has not been established using *in vivo* preparations. Another approach is to use clonal cell lines derived from the CNS, for their cellular homogeneity facilitates the assay of pool sizes of neurotransmitters and free amino acids in identified cells. Such determinations have been made with fibroblasts[5-7].

The expanding collection of clonal cell lines from the CNS[8-11] and other tissues (for example, refs 12-14) presents the opportunity to extend this approach. We describe here the free amino acid pools of some cell lines from the CNS and other tissues which exhibit differentiated phenotypes. We have found that although several specific differences exist between cells from different tissues, there is a strong similarity between different cell types of common embryological origin.

We used the rat nerve cell lines B35, B50, B65, B103 and B104, and the rat putative glial cell lines B11, B12, B15, B23, B28, B49, B82, B90, B92 and B108 (ref. 11), and two other lines of glial origin, C6 (ref. 8) and RN2 (ref. 15). Additional clonal cell lines examined were: smooth muscles BC_3H1 (ref. 16) and A10 (B. Kimes and B. Brandt, submitted); skeletal muscle L6 (ref. 13); plasmacytoma C1 and lymphomas S1A and S49 (ref. 14); neurblastoma clones C1 (ref. 10) and N18 (ref. 17); melanomas[18] B78 (non-pigmented) and B559 (pigmented); hepatoma H35 (ref. 19); growth hormone and prolactin-producing pituitary cells GH_3 (ref. 20); adrenal cortical line Y1 (ref. 12), and the fibroblast cell line 3T3 (ref. 21). All cells were grown in modified Eagle's MEM containing 10% foetal calf serum[11].

Early stationary phase cultures, 2 or 3 d after cessation of net cell division, were used exclusively for the determination of free amino acids, for cells at this stage of the growth cycle usually express maximum morphological and biochemical differentiation[22,23]. Cell viability was greater than 95% as measured by the uptake of fluorescein dibutyrate[24].

Free amino acids were extracted from cells washed four times in phosphate buffered saline (PBS, 150 mM sodium chloride, 10 mM sodium phosphate, pH 7.3, 2 mM calcium chloride, 0.5 mM magnesium chloride) according to a modification of the trichloroacetic acid (TCA) procedure of Saifer[25]. Frozen cell pellets were homogenised at 0 °C with 20 volumes of 6% TCA using a Duall tissue grinder and centrifuged at 12,000g for 20 min. The procedure was repeated on the TCA precipitate, and the two supernatants were combined. A third extraction of the TCA precipitate yielded no more detectable free amino acids (less than 3% of the combined previous two extracts). The TCA was then extracted four times with 3 volumes of ethyl ether, the aqueous extract was lyophilised and dissolved in the starting buffer for the amino acid analysis. Amino acids were analysed with a Beckman amino acid analyser, Model 121, equipped with an automatic integrating device and run with an expanded elution profile[26]. More than 40 amino acids and related compounds are resolved by this procedure. Since cell volume and protein per cell varies with chromosome number and growth phase (for example, ref. 27), the data are expressed as residues of a particular molecule per 1,000 residues of resolved free amino acids and related compounds.

GABA, glutamic acid, glycine, aspartic acid, taurine, (reviewed, for example in refs 28-30) and β-alanine[31] are putative neurotransmitters in the CNS. Although little is known about their relative distribution between nerve, glia and other differentiated cells, clonal cell lines can be used to study their synthesis and/or accumulation. Table 1 shows the relative concentrations of the putative CNS transmitters in several cell types. There is little statistical difference between the GABA content of nerve and glial cells, although individual nerve lines contain much more GABA than observed in any glial line. The former is also true with the other putative neurotransmitters. The GABA pools do not necessarily correlate with glutamic acid decarboxylase activities[11,23], for the retention of amino acids was assayed, not synthetic ability. The only qualitative difference between cell types was the lack of detectable GABA and β-alanine in muscle and fibroblast cells.

Table 1 Relative concentrations of putative CNS neurotransmitters in clonal cell lines

Cell line	Cell type	GABA	β-ALA	GLU	ASP and ASPN	GLY	TAU
				Relative concentration (residues per 1,000 free amino acids ± s.d.)			
B35	Nerve	7.1±0.3 (3)	35±6 (3)	203±6.5 (3)	53±5 (3)	235±17 (3)	207±19.7 (3)
B50	Nerve	30.0	75.2	153	7.6	283	202
B65	Nerve	75.1±13.1(5)	51.6±4.2(5)	144±10.1(5)	21±0.7 (5)	203±7.8(5)	123±9.2(5)
B103	Nerve	7.3±0.1(3)	20.6±2.7(3)	104±6.5(3)	46±2.9 (3)	313±7.5(3)	96.1±11.8(3)
B104	Nerve	5.9	11.2	143	56.7	108	86.1
N18	Nerve	9.0	13.8	114	42.7	269	157
Nb-C1	Nerve	4.2	8.6	28.4	20.3	265	126
B11	Glial	13.4	35.8	171	49.7	144	190
B12	Glial	2.1	21.1	155	57.5	135	70.6
B15	Glial	1.5	9.3	103	105	96.2	67.5
B23	Glial	5.6	93.2	274	50.4	112	168
B28	Glial	19.5	48.1	212	52.7	153	240
B49	Glial	4.8	50.6	148	33.4	96.5	184
B82	Glial	1.1	2.3	84.0	85.7	90.7	28.8
B90	Glial	1.4	10.2	194	58.3	119	117
B92	Glial	2.1	4.0	178	69.2	65.9	22.9
B108	Glial	3.4	20.0	217	66.6	154	146
C6	Glial	15.5	3.3	309	65.4	163	30.9
RN2	Glial	4.4	38.7	156	20.1	227	127
B78	Melanocyte	6.1	26.4	48.7	18.6	329	71.8
B559	Melanocyte	3.5	5.5	50.5	54.9	195	41.3
S1A	Lymphocyte	1.4	30.1	172	25.7	281	209
C1	Plasmacyte	1.7	10.1	295	74.9	134	154
S49	Lymphocyte	1.7	14.2	198	34.2	250	152
Y1	Adrenal cortical	6.5	7.9	58.2	64.2	188	85.4
GH_3	Pituitary	7.2	4.7	184	37.1	166	160
L6	Skeletal muscle	<0.1	<0.1	120	65.8	123	50.5
BC_3H1	Smooth muscle	<0.1	<0.1	64.0	81.3	99.2	137
A10	Smooth muscle	<0.1	<0.1	19.8	59	63	13.5
3T3	Fibroblast	<0.1	<0.1	59.0	78.5	82.6	68.6
H35	Hepatocyte	<0.1	<0.1	80.9	72.6	240	38.8
Serum		<0.1	3.5	80.6	60.5	59.3	16.7
Medium		<0.1	<0.1	<0.1	<0.1	8.4	<0.1

Free amino acids were extracted from cells by TCA precipitation and chromatographed on an amino acid analyser. Serum and medium samples were treated in an identical manner. The sum of the aspartic acid and asparagine peaks are given. Where indicated, the data are presented as the mean ± (s.d.) for n determinations using different cell preparations. Abbreviations: GABA, γ-aminobutyric acid; β-ALA, β-alanine; GLU, glutamic acid; ASP, aspartic acid; ASPN, asparagine; GLY, glycine; TAU, taurine.

Of the remaining amino acids, there were no significant differences between the different cell types. Finally, several unidentified, ninhydrin-reacting compounds eluted at times which did not correspond to the standards. For example, cells contained compounds which eluted between DL-O-phosphoserine and O-phosphoethanolamine, and between urea and hydroxyproline. These compounds never individually constituted more than a few residues per thousand, and were found in similar amounts in all cells.

The relative concentrations of free amino acids described in the nerve and glial cell lines are in general agreement with those published for whole brain (for example, ref. 25), although there are exceptions. For example, the concentration of glutamic acid was the highest of the free amino acids in whole cerebral tissue[25] and the nerve and glial cell lines (Table 1), but the concentration of aspartic acid was significantly higher in the brain than the cell lines (Table 1). Such discrepancies are, however, to be expected since the multiplicity of cell types found *in vivo* may not have been represented among the clonal cell lines.

Table 1 shows that cells derived from neuroectoderm contained detectable amounts of GABA and β-alanine, although the mesodermally derived muscle and fibroblast cells did not. Thus nerve, glia, melanocytes and neuroblastoma clones had qualitatively similar free amino acid pools distinct from those of muscle and fibroblast cells. It has been shown that nerve and glial cells share a number of additional traits distinct from muscle, such as acetylcholine and catecholamine synthesis, GABA uptake, S100 and 14-3-2 protein synthesis, and a morphological response to cyclic nucleotides[11,32,33]. Other cell types, such as those derived from lymphoid tissue, the adrenal cortex and the anterior pituitary, also contained both GABA and β-alanine (Table 1). Thus GABA and β-alanine were excluded from most mesodermal tissues as opposed to being uniquely associated with nervous tissue. The apparent exception was the adrenal cortical clone, which is thought to be of mesodermal origin, but contained GABA (Table 1). The functional similarity between cells of the immune and nervous systems has, however, been pointed out repeatedly (for example, ref. 34). In addition, GABA has been found in other tissues in smaller amounts than in the nervous system and may serve a metabolic role (for example, ref. 35). Since no GABA was detected in the culture medium, cells containing GABA probably synthesise it. Although it is likely that these cells also synthesise β-alanine, this is not proven, for serum contained this compound (Table 1) and it may be concentrated. It should be noted, however, that cells contain both GABA and β-alanine, or neither, probably because glutamic acid decarboxylase can use both aspartic and glutamic acids as substrates to produce β-alanine and GABA, respectively[36].

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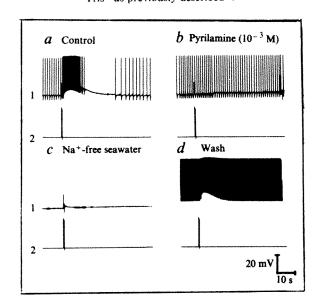
H₁ and H₂ histamine receptors on *Aplysia* neurones

WE have found two types of histamine receptor on neurones of the mollusc *Aplysia* corresponding to those already known in mammals. The presence of these specific receptors is consistent with the suggestion that histamine acts as a neurotransmitter.

Histamine, originating primarily from mast cells, is an important pharmacological agent in the regulation of smooth muscle and visceral functions in mammals[1]. Two types of histamine receptor, H₁ and H₂, have been described. Activation of H₁ results principally in smooth muscle contraction and is blocked by pyrilamine and several other compounds. H₂ mediates stimulation of gastric secretion, increases in heart rate and inhibition of uterine contraction, and its response is blocked by burimamide but is insensitive to H₁ blocking drugs[2,3].

Although it seems likely that histamine functions as a neurotransmitter in the central nervous system (CNS), there is no definitive evidence for this. Some cortical neurones of the cat respond to iontophoretic histamine by both depression and excitation[4], and a relatively high proportion of hypothalamic

Fig. 1 Responses of an unidentified neurone from the left pleural ganglion to 1,000-nC pulses of histamine. Trace 1 is the intracellular recording and trace 2 indicates the iontophoretic pulse. Pyrilamine was dissolved in seawater and perfused over the ganglion. Na⁺ replacement was made with Tris⁺ as previously described[14].

neurones is excited by histamine[5]. It has been suggested, on the basis of postdegenerative changes in local histamine concentrations, that histaminergic pathways run through the medial forebrain bundle[6]. Histamine affects sympathetic ganglia, but it is not clear whether it functions in this system as a neurotransmitter or simply modulates other transmitter systems[7,8].

Previous work on an invertebrate (*Helix*) nervous system has demonstrated that both excitatory and inhibitory responses can be recorded when histamine is added to the bath and that both kinds of response are blocked by pyrilamine[9]. We have extended these observations, applying the drug iontophoretically, testing both H_1 and H_2 blocking drugs and determining which ionic conductances are changed by histamine.

Histamine is present in most identified neurones of *Aplysia* in concentrations of 10^{-6}—10^{-5} M (ref. 10). Weinreich *et al.*[11] have found two small neurones in the cerebral ganglion containing histamine in concentrations as great as 4×10^{-4} M. While surveying for the presence of receptors for several amines, we have found two types of response to histamine, the pharmacological sensitivities of which correspond to those of mammalian H_1 and H_2 receptors.

Experiments were performed on pleural, pedal, cerebral and abdominal ganglia of *Aplysia californica* removed from the animal and maintained under flowing artificial seawater in a Lucite chamber. As before[12], neurones were penetrated with double-barrelled glass pipettes for recording and electrical stimulation. Putative neurotransmitters were applied iontophoretically, through a five-barrelled glass pipette controlled by an electronic unit which delivered constant charge rather than current. Histamine was placed in one of the barrels at a concentration of 1 M, pH 3.5.
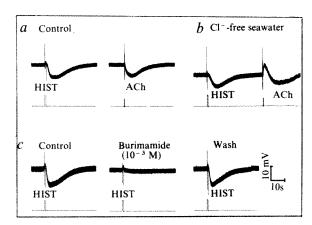
Fig. 2 Response of an unidentified cerebral neurone to histamine (HIST) and acetylcholine (ACh), 500-nC pulses. For (*b*) the preparation was perfused with a solution in which all Cl⁻ salts were replaced by isosmotic acetate salts. Burimamide was dissolved in seawater and perfused over the preparation.

Receptors for histamine were found on only a small fraction of neurones, and when present appeared both on the cell body and in the neuropile. All receptors were specific for histamine and showed no sensitivity to acetylcholine, dopamine, octopamine, phenylethanolamine or noradrenaline. Figure 1 shows the response of an unidentified neurone in the left pleural ganglion to a 1,000-nC pulse of histamine. After a very short latency, histamine caused a marked depolarisation and increase in discharge. The threshold for response was 500 nC. Pyrilamine (10^{-3} M), an H_1 blocking agent, reversibly abolished the response after exposure for 10 min (Fig. 1*b*). After recovery to control, the ganglion was perfused with seawater in which all Na⁺ was replaced with Tris⁺, which is at least relatively impermeable in this preparation. As Fig. 1*c* shows, the response was totally blocked in Tris⁺ seawater. This and all other responses to histamine which we have observed have been associated with an increase in

membrane conductance. Because both the voltage shift and the conductance change were absent in Na⁺-free seawater, this response seems to be the result of an increase in only Na⁺ conductance. The absence of action potentials in Fig. 1*c* results from an independent Na⁺ requirement. All these manipulations were reversible (Fig. 1*d*). This response was unaffected by exposure to 10^{-3} M burimamide for 20 min.

Figure 2 is from studies on an unidentified cerebral neurone with a different type of response. Figure 2*a* shows that the neurone responded with a slow hyperpolarisation to 500-nC pulses of both histamine and acetylcholine. An increased membrane conductance was measured during both responses. When all Cl⁻ in the seawater was replaced by the impermeant anion acetate, the membrane potential increased by about 7 mV and, whereas the histamine response remained essentially unchanged, the acetylcholine response became biphasic. The biphasic response resulted because acetylcholine caused an early increase in conductance of Cl⁻ followed by a late increase in K⁺ conductance[13]. The Cl⁻ component was depolarising in acetate seawater because the Cl⁻ equilibrium potential was then more depolarised. Because the histamine response did not change in acetate seawater, we assume that it, like the late acetylcholine response, resulted from a conductance increase to K⁺. Burimamide, the H_2 blocker, reversibly abolished this response in less than 5 min, but pyrilamine (10^{-3} M) had no effect after exposure for 30-min.

All histamine responses recorded were sensitive to either pyrilamine or burimamide, but not both. Both depolarising and hyperpolarising responses were found sensitive to each drug. These observations suggest that in *Aplysia* there are two types of histamine receptor corresponding to the H_1 and H_2 receptors described for mammals[2,3], and that each receptor is coupled to either an Na⁺ or a K⁺ conductance mechanism. Although identification of a substance as a neurotransmitter is absolutely dependent on demonstration of its release on nerve stimulation, and this has not been shown for histamine in this population of neurones, the presence of sensitive and specific receptors is consistent with a neurotransmitter function of histamine.

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Evidence for neuronal control of ion transport in chironomid larvae

THE role of anal papillae in the uptake of sodium and chloride ions has been established in the closely related culicid and chironomid larvae[1-6], but studies on the ultrastructure of these organs have been limited to the former group[7-9]. The sites of active ion transport are located on the apical membrane of the epidermal cells below the cuticle[5,8,10]. The mechanism by which ion transport is controlled in culicid larvae is most

probably hormonal[4,11] but there are indications that a different system operates in the chironomid larvae[12-14]. To investigate this possibility, the ultrastructure of the anal papillae of *Chironomus riparius* Meigen has been examined. Axons were clearly present suggesting that ion transport may be under neuronal control.

The basic structure of the anal papillae of larval *C. riparius* is very similar to that described in mosquito larvae (Fig. 1). The cuticle is thin, relative to that over much of the rest of the body, and its surface is thrown into complex waves. The apical membrane of the epidermal cells is deeply infolded and numerous mitochondria are located adjacent to the proximal limit of these folds. The basal membrane is also irregular, leaving channels penetrating almost to the limit of the apical membrane. A number of 'mitochondrial pumps' similar to those described by Copeland[7] in the corresponding region of *Culex quinquefasciatus* are situated among the intrusions of the basal membrane.

Two features distinguish the anal papillae of this chironomid species from those of mosquitoes. Tracheae and tracheoles are absent, but groups of axons are present in both the dorsal and ventral papillae of the chironomid. No axons have been reported in this region of any mosquito.

The examination of entire papillae, whether removed from the animal or not, did not reveal the presence of any nervous tissue. Similarly, conventional sectioning of wax-embedded papillae did not show any detail of the nerves. The use of silver staining on whole or sectioned tissue was also fruitless as the high concentration of Cl^- resulted in deposition of silver throughout the papilla[15]. The identification of axons has therefore been based exclusively on their appearance in thin sections examined in the electron microscope, where they are seen to contain microtubules, the diameter of which (about 20 nm) is compatible with that of typical neurotubules[16] (Fig. 1).

Fig. 1 The integument of the anal papilla of a larva of *C. riparius*, seen in thin section stained with uranyl acetate and lead citrate. Note the irregular membranes and small nerve. Distally, the individual axon in contact with the epidermis becomes invested in folds of the basal membrane. (×9,350)

The number of axons in each papilla is variable, but when sectioned transversely at their proximal end the number in each can be estimated. At this level there are usually twelve to fourteen axons in each of the ventral papillae and seven to ten in each dorsal papilla. As the degree of branching is unknown, closer estimates have not been attempted. The axons are grouped into two small nerves, diametrically opposed across the papilla, with occasional isolated axons separated from the main bundles. At this level in the papilla the axons are not always in contact with the epidermis. The source of the axons is not yet known.

Distally the number of axons or their branches is smaller, and individual axons can be seen penetrating the epidermis. They never seem to become intracellular but become closely

invested in folds of the basal membrane. Terminally, the axons increase in diameter and a swelling is formed that contains numerous vesicles, the diameter of which has been calculated as 80 ± 3 nm (ref. 17). The swelling is extracellular but located at the distal limit of the basal membrane near the mitochondria and apical membrane. No sense organs or cell bodies have been seen associated with these axons in the anal papillae and it is therefore presumed that they do not serve a sensory role.

The function of the axons and any transmitter which may be released from the vesicles at their termination is not yet known. If their existence, however, is related to Koch's observation that anticholinesterases inhibit net sodium transport[12], it may be concluded that the axons serve some role in the control of active ion uptake in the papillae of *Chironomus* larvae. As no axons have been reported in the papillae of mosquito larvae (nor could they be seen on examination of the published evidence) this conclusion would not contradict the idea of hormonal regulation of this activity in the mosquito *Aedes aegypti*[11]. Nevertheless, it would emphasise that parallels between ionic regulation in culicid and chironomid larvae should be drawn with great care. Certainly, there may be differences in the sodium transporting mechanisms located in the anal papillae of these groups.

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Biosynthesis of γ-aminobutyric acid by isolated axons of cone horizontal cells in the goldfish retina

SPECIFIC proteolytic enzymes have been used to dissociate adult vertebrate retinae into single cells[1-3]. These methods facilitate the study of chemical and physiological properties of isolated, identified retinal cells. In particular, the synthesis of neurotransmitter candidates in a pure retinal cell type has been studied by drawing identified cells into a micropipette, incubating them with appropriate radioactive precursors, and analysing the presence of radioactive transmitters[1]. In the study reported here, this method was used to examine transmitter synthesis by horizontal cells. Cajal's internal horizontal cells[4] from the goldfish retina were used because they could be dissociated easily from the retina and identified readily. In addition, these cells have been shown to be connected to Cajal's external horizontal cells, and can therefore be considered as fusiform axon terminals of cone horizontal cells[5,6].

Before studying transmitters in dissociated cells, transmitter candidates synthesised by the whole goldfish retina were examined by the method of Hildebrand *et al.*[7]. As
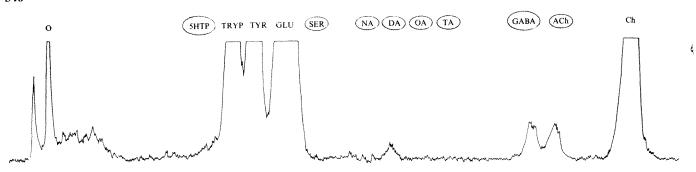
Fig. 1 Radiochemical scanning of an electrophoretogram from a homogenate of goldfish retina incubated with ¹⁴C-labelled tryptophan, tyrosine, glutamic acid and choline chloride for 1 h. Two retinae (about 100 mg tissue weight) from an adult goldfish (*Carissius auratus*), were isolated and incubated with 1.0 ml of isotonic Leibovitz medium[8] (L-15, Grand Island Biological Co.) containing 10 μCi of each of methyl-¹⁴C-choline chloride (30 Ci mol⁻¹, Ch), L-U-¹⁴C-glutamic acid (255 Ci mol⁻¹, Glu), L-U-¹⁴C-tyrosine (54 Ci mol⁻¹, Tyr) and L-methylene-¹⁴C-tryptophan[7,9] (54.5 Ci mol⁻¹, Tryp). After 0.5 to 2.0 h of incubation at 24 °C, the retinae were homogenised with twice the tissue weight of formic acid (0.47 M)–acetic acid (1.4 M), and the homogenate was analysed by high voltage electrophoresis (6,000 V, 2 h) for the synthesis of ¹⁴C-ACh, GABA, DA, noradrenaline (NA), octopamine (OA), tyranine (TA), 5-hydroxytryptophan (5-HTP) and serotonin (SER). Scale: origin (O) to Ch is 72 cm.

Fig. 1 shows, of all the transmitter candidates examined in this study, goldfish retinae synthesise only acetylcholine (ACh), γ-aminobutyric acid (GABA) and dopamine (DA). ACh and GABA synthesised were identified by specific enzymatic degradation[5,10] and ascending paper chromatography[11,12]. DA was also identified by paper chromatography[11]. Furthermore, significant activities of the
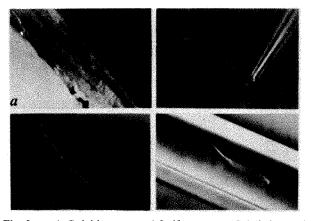
Fig. 2 *a*, A Golgi-impregnated fusiform axon (Cajal's internal horizontal cell) from the goldfish retina; *b*, an isolated fusiform axon from the goldfish retina; *c*, a fusiform axon drawn into a micropipette; *d*, a fusiform axon in a micropipette during incubation. (×175, Nomarski interference optics). The medium used for cell dissociation was modified L-15, made up of regular L-15 medium supplemented with glucose (2 g l⁻¹), ethyleneglycol *bis*(aminoethyl) tetraacetic acid (EGTA, 5 mM), penicillin (1,000 U ml⁻¹), streptomycin sulphate (0.5 mg ml⁻¹) and diluted to a tonicity of 250 mosmol, pH 7.2. In each experiment two to four goldfish retinae (100–200 mg) were incubated at 24 °C for 2 h with gentle shaking in 3.0 ml of sterile, oxygenated modified, L-15 medium containing crystalline papain (EC 3. 4. 4. 10) (1.0 mg ml⁻¹, 11.2 U mg⁻¹, Sigma). Retinae were then washed for 3 min with 5.0 ml modified L-15 medium and transferred to a conical tube containing 3.0 ml modified L-15 medium supplemented with 0.5% bovine serum albumin (fraction V, Sigma). Cells were dissociated by gently stirring and pipetting retinae up and down through a wide-bore Pasteur pipette. After the suspension had settled for 5 min, 2.0 ml was taken from the top fraction and filtered through a sterile gauze pad to remove cell clumps. The filtrate was then applied to a 40-ml velocity sedimentation chamber (made from a 50-ml plastic syringe) for cell separation[1]. Cells were separated by unit gravity at 24 °C through a linear gradient of 1–3% bovine serum albumin in modified L-15 medium. After 2–3 h of sedimentation, 2.0-ml fractions were collected and examined using a haemocytometer. A 0.1-ml sample of a fraction rich in these axons was spread on a glass slide. Under Nomarski interference optics (×256), single axons were drawn into a glass micropipette with a tip of inner diameter 6–8 μm attached to a micromanipulator (*c*). About 100 axons were drawn into each micropipette and used for chemical analysis.

transmitter-synthesising enzymes choline acetyltransferase (EC 2. 3. 1. 6), glutamate decarboxylase (EC 4. 1. 1. 15), and tyrosine hydroxylase (EC 1. 14. 3a) in cell-free extracts of goldfish retina can be demonstrated (ref. 10 and D.M.K.L. unpublished).
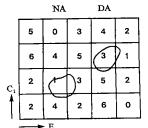
To examine transmitter candidates synthesised by fusiform axons of cone horizontal cells[6] (Cajal's internal horizontal cells[4]) from the goldfish retina, a pure population of these axons was prepared by a modified cell dissociation and separation method[1]. By comparing the dimensions of the dissociated cells (Fig. 2*b*) with Golgi-impregnated fusiform axons of cone horizontal cells from the goldfish retina (Fig. 2*a*), isolated fusiform axons could be recognised easily and drawn into a glass micropipette individually (Fig. 2*c* and *d*) under visual observation. Figure 2 also shows that the isolated fusiform axons did not have nuclei, a finding consistent with earlier histological observations[13].

A hundred isolated fusiform axons were drawn into a micropipette, incubated with H³-labelled precursors and analysed for transmitter synthesis. Results of analyses from a typical experiment showed that these fusiform axons synthesised and accumulated GABA, but no detectable ACh and DA (Fig. 3).

These results did not show whether the level of GABA synthesis by these axons was significant compared with that in the whole retina. To obtain this information the enzymatic activity of glutamate decarboxylase in extracts of these axons was measured. A hundred fusiform axons were drawn into a micropipette and lysed by repeated freezing and thawing with dry ice. Both the glutamate decarboxylase and choline acetyltransferase activities in isolated axons and whole retinae were measured as described earlier[1,9,10]. After a 3 h incubation, radioactive products were separated and identified by two-dimensional paper electrophoresis and chromatography[11]. To calculate specific enzymatic activity, it is necessary to know the tissue weight of protein content. From microscopic measurements, the isolated fusiform axons used in this study had an average length of 100–200 μm and diameter of 3–6 μm. The specific activity of glutamate decarboxylase in these axons was calculated by assuming that an axon had a volume similar to a cylinder of radius 1.7 μm and 100 μm long, giving an approximate volume of 9×10^{-8} ml or a weight of 9.4×10^{-8} g per axon. On this basis the specific activity of glutamate decarboxylase in the isolated fusiform axons was 1.2 ± 0.4 μmol GABA formed h⁻¹ g⁻¹ wet weight, a value similar to that measured in the whole retina (1.0 ± 0.1, ref. 10). The specific activity of choline acetyltransferase in isolated fusiform axons (<0.05 μmol ACh formed h⁻¹ g⁻¹ wet weight) was below the sensitivity of this assay and was less than 5% of that

measured in the goldfish retina ($1\,8\pm0\,3$, D M K L , unpublished)

If GABA is to be considered a transmitter candidate in cone horizontal cells, the specific activity of glutamate decarboxylase in them should be substantially higher than that in the whole retina So, does the specific activity measured in isolated fusiform axons represent that in entire cone horizontal cells, which consist of both cell bodies and axons? Because isolated cone (external) and rod (intermediate) horizontal cell bodies could not be distinguished unequivocally, enzymatic activities in the cell bodies could not be measured directly Since in cone horizontal cells of the cyprinid retina, however, most of the structures characteristic of the chemical synapse have been observed on the cell bodies and not on the fusiform axons[3,14 15], the isolated axons used in this study probably contained very few synaptic endings (Fig 2) As glutamate decarboxylase has been shown to be very concentrated in presynaptic endings of neurones which presumably use GABA as a transmitter[16,17], the specific activity of glutamate decarboxylase in a cone horizontal cell complete with all its synaptic endings is probably much higher than that in an isolated
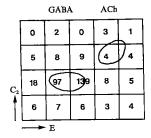
Fig 3 Two-dimensional analyses of radioactive transmitters synthesised by 100 fusiform axons incubated with purified methyl-³H-choline chloride (6 7 Ci mmol⁻¹), and L-³H-glutamic acid (generally labelled, 2 5 Ci mmol⁻¹) and L-3, 5-³H-tyrosine (25 Ci mmol⁻¹) for 8 h at 24 °C After incubation, cells were lysed by drawing 1 0 μl of 1 M HCl containing unlabelled precursors and transmitters into the micropipette Radioactive products were first separated by paper electrophoresis Regions corresponding to noradrenaline (NA) and dopamine (DA) were analysed further by ascending paper chromatography using a solvent system C₁ methylethyl ketone–propionic acid–water (200 65 55)[11] Regions corresponding to GABA and ACh were similarly analysed using another solvent system C₂ n-butanol–acetic acid–water (60 15 25)[11,12] Each chromatogram was dried, stained and cut into 1-cm squares Each square was placed in a scintillation vial containing 1 ml 0 01 M HCl for 1 h 9 ml of Aquasol was then added to each vial and the radioactivity was measured by scintillation counting[18] (25% efficiency) The values represent net c p m above a background of about 19 c p m

fusiform axon It is therefore reasonable to assume, on chemical and morphological grounds (Fig 2), that the specific activity of glutamate decarboxylase in intact cone horizontal cells of the goldfish retina is substantially higher than that in the whole retina

Lam and Steinman have shown that in the goldfish retina, a large amount of ³H-GABA is accumulated selectively by cone horizontal cell bodies and fusiform axons (Cajal's external and internal horizontal cells)[18] In addition, both the accumulation of ³H-GABA into these cells and the endogeneous level of GABA in the retina were influenced light stimulation of the retina[16,23] This study complements those results and shows that besides having a mechanism to accumulate exogenous GABA selectively, cone horizontal cells also possess the machinery to synthesis GABA These findings point to GABA as a transmitter candidate used by cone horizontal cells of the goldfish retina

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β-Adrenergic stimulation of cyclic AMP and protein kinase activity in the thyroid

THE demonstration that the sympathetic nervous system can have a direct effect on the thyroid[1], and the increasing clinical use of adrenergic blocking agents in thyrotoxicosis have reawakened interest in the effects of catecholamines on the thyroid We have therefore compared the effects of thyroid stimulating hormone (TSH) and isoproterenol, a β-adrenergic stimulator, on the activation of protein kinase within the thyroid by cyclic AMP We have also compared the inhibitory effects of the β-blocker propranolol, and the α-blocker phentolamine in this system We found that isoproterenol is stimulatory at concentrations as low as 10^{-8} M, and that l-propranolol, but not d-propranolol blocks this effect

Slices of calf thyroid were incubated and then frozen as before[2] Intracellular cyclic AMP was determined by the method of Steiner et al[3], and expressed per mg of protein in the homogenate Intracellular protein kinase activity in the frozen powdered tissue was determined as before[2] using a 2 mM EDTA/5 mM KHPO₄ homogenising buffer, pH 7 0, to which 0 5 mM 1-methyl-3-isobutyl xanthine and 200 mM NaCl had been added The ratio of the activity without added cyclic AMP to the activity found when 1 μM cyclic AMP is added during the assay reflects the degree of intra-

Table 1   Effect of TSH and isoproterenol on cyclic AMP and protein kinase activity

	Cyclic AMP (pmol mg⁻¹)	Activity ratio
Control	5 6±0 5	0 33±0 04
dl-Isoproterenol (5×10⁻⁵ M)	16 ±1 0*	0 63±0 03‡
TSH (50 mU ml⁻¹)	65 ±8†	0 99±0 04§
Isoproterenol +TSH	82 ±7	1 03±0 05

Means ±s e m , n = 4 The values for TSH alone are not significantly different from those when isoproterenol was added to TSH
*P<0 005 compared with control ·
†P<0 01 compared with control and <0 01 compared with isoproterenol alone
‡P<0 025 compared with control
§P<0 001 compared with control and <0 01 compared with isoproterenol alone
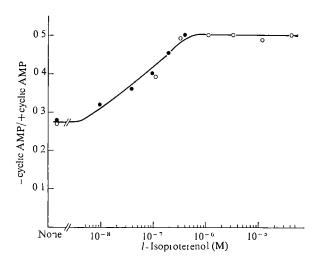
Fig. 1 Dose–response Slices were incubated with various concentrations of *l*-isoproterenol for 10 min A 20,000*g* supernatant from tissue homogenate was assayed for its ability to incorporate ^{32}P from ATP into mixed calf thymus histone The 'activity ratio', the ratio of activity without added cyclic AMP to activity with 1 μM cyclic AMP added to the assay reflects the degree of intracellular activation of the enzyme ○, Results from one experiment, performed in duplicate, ●, results from a second experiment, also performed in duplicate

cellular activation of protein kinase We refer to this measure of endogenous activation as the 'activity ratio'

Thyroid slices were incubated with *l*-isoproterenol for various times The activity ratio increased linearly from zero time to 2 min, then remained constant for at least 10 min Subsequent incubations were carried out for 10 min, which was the time previously found for TSH at 50 mU ml^{-1} to produce its maximal effect in this system[2] Figure 1 shows the response of the kinase activity ratio to various concentrations of *l*-isoproterenol Concentrations as low as 10^{-8} M produced a significant increase in the activity ratio, and half maximal stimulation occurred at approximately 7×10^{-8} M

Table 1 shows the relative effects of large doses of *dl*-isoproterenol (5×10^{-5} M) and TSH (50 mU ml^{-1}) on cyclic AMP and kinase activity (Table 1) TSH fully activated the kinase activity ratio (0 99), while isoproterenol, which only raised the cyclic AMP level to 16 pmol mg^{-1}, increased the kinase activity ratio to 0 63 The cyclic AMP level produced when isoproterenol and TSH were combined was not significantly greater than that produced by TSH alone

To characterise the receptors involved, thyroid slices were preincubated with the β-adrenergic receptor blocking agent propranolol for 15 min Control slices contained $6 8 \pm 0 6$ pmol mg^{-1} cyclic AMP and this level was unaltered by preincubation with 10^{-4}M *dl*-propranolol TSH at 16 mU ml^{-1}, a submaximally stimulatory dose, raised the cyclic AMP concentration to 29 ± 5 pmol mg^{-1}, which was also unaffected by propranolol Isoproterenol (5×10^{-5} M) raised

Table 2 Effect of *d*-and *l*-propranolol on the isoproterenol-mediated stimulation of cyclic AMP and protein kinase activity

	Cyclic AMP	Activity ratio
Control	6 0±0 4	0 28±0 02
l-Isoproterenol (6×10^{-7} M)	11 7±1 0*	0 42±0 02*
Isoproterenol + *d*-propranolol (6×10^{-6} M)	15 9±1 0†	0 51±0 02†
Isoproterenol + *l*-propranolol (6×10^{-6} M)	8 1±1 0†	0 32±0 01‡

*$P < 0 005$ compared with control
†$P < 0 05$ compared with isoproterenol alone
‡$P < 0 005$ compared with isoproterenol alone
Isoproterenol +*l*-propranolol was not significantly different from control

the cyclic AMP concentration to $16 8 \pm 1 4$ pmol mg^{-1}, while propranolol reduced this response to $12 3 \pm 1 0$ ($P < 0 025$) The kinase activity ratio in the control slices in this experiment was $0 42 \pm 0 01$ and was unaltered by propranolol *dl*-Isoproterenol (5×10^{-5} M) raised the activity ratio to $0 64 \pm 0 03$ and this was reduced by propranolol to $0 50 \pm 0 03$ ($P < 0 005$) Preincubation of the slices with 10^{-4} M phentolamine, an α-adrenergic blocking agent, had no effect on the stimulation produced either by TSH at mU ml^{-1} ($0 81 \pm 0 04$) or 5×10^{-5} M isoproterenol

Low concentrations of the purified isomers of propranolol were also compared for inhibitory effects on the stimulation produced by *l*-isoproterenol (Table 2) While 6×10^{-6} M *l*-propranolol significantly lowered the cyclic AMP and kinase activity ratio, preincubation with *d*-propranolol actually enhanced the response to isoproterenol The kinase activity ratio and the intracellular cyclic AMP concentrations correlated closely (Fig 3)

In 1968, Gilman and Rall suggested that β rather than α-adrenergic receptors mediated the effects of adrenaline in bovine thyroid slices[4] Subsequent studies on adrenergic stimulation of the thyroid have, however, been confusing
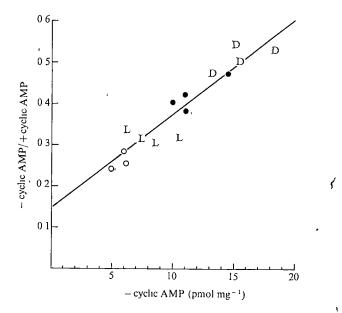
Fig 2 Correlation of kinase activity and intracellular cyclic AMP Slices were preincubated with *d*- or *l*-propanolol (6×10^{-6} M) for 15 min then incubated with 6×10^{-7} M *l*-isoproterenol for 10 min The two isomers of propranolol had no significant effects in the absence of the isoproterenol Correlation coefficient $r = 0 93$, activity ratio $= 0 023$ (cyclic AMP) $\pm$ 0 146 ○, Control slices, ●, slices incubated with isoproterenol, D indicates preincubation with *d*-propranolol and L indicates *l*-propranolol

and apparently contradictory, but have been generally interpreted as indicating the adrenergic effects on the thyroid were mediated by α receptors[5-8] The adrenergic blocking agents phentolamine and propanolol at concentrations greater than 10^{-4} M have even been reported to inhibit the effects of TSH on adenyl cyclase in thyroid slices[8-11] These findings have been interpreted as a reflection of a direct pharmacological effect on membranes by these blocking agents[11] In this study, however, only *l*-propranolol was inhibitory, while both *d*- and *l*-propranolol exert membrane effects The apparently synergistic effect of *d*-propranolol, while unexpected, is not unprecedented, as stimulatory effects of *d*-propranolol have been observed previously[12]

We have found previously that the activation of cyclic AMP-dependent protein kinase correlates well with increases in the level of intracellular cyclic AMP produced by TSH and prostaglandin E_1 in calf thyroid slices[2,13] Although the magnitude of the response of the activity

ratio to β-adrenergic stimulation is clearly less than that produced by TSH, it is apparently faster, reaching its maximum effect within 2 min, as opposed to about 10 min for TSH[2] Adrenaline was reported to produce a half maximal response in cyclic AMP at a concentration of 3×10^{-7} M in calf thyroid slices[4], although iodide uptake could be stimulated by as little as 5×10^{-7} adrenaline in isolated bovine thyroid cells[5] Our observation of an increase in kinase activity with as little as 10^{-8} M isoproterenol suggests that lower concentrations of this pure β stimulator are required than are needed for an effect of the combined α and β stimulator, adrenaline

Comparison of the action of isoproterenol and TSH reveals that isoproterenol acts more rapidly but does not raise cyclic AMP or stimulate kinase activity to the same degree as TSH These findings, as well as the lack of effect of propranolol on TSH suggest that the two agents act on different receptor sites The inhibition of the kinase response to isoproterenol by *l*- but not *d*-propranolol, as well as the lack of effect of phentolamine, indicates that the isoproterenol effect is mediated by β receptors

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Influence of the pineal on wound healing

REMOVAL of the pineal gland affects endocrine functions in both birds and mammals, and has been shown to cause increased growth and spread of malignant tumours in the rodent There are several hypotheses as to the mechanism by which the pineal affects these processes[1-4], but no role for the gland or its hormonal product melatonin has been suggested in normal processes We have now found that pinealectomy can slow wound healing and that this effect can be reversed by treatment with melatonin

Twenty 4-week-old inbred C57BL mice were divided into four groups of five The mice in group 1 were pinealectomised, group 2 was sham-operated, group 3 received melatonin (1 mg kg^{-1} body weight) intraperitoneally daily, beginning 24 h after pinealectomy and continuing for 10 d, and group 4 served as untreated controls All animals were kept in transparent plastic cages in a 10×12 foot room at 74–78 °F (23–26 °C) with 40% relative humidity A 60-W bulb provided light during the day, and was turned off at night The animals were fed Rockland hamster diet and water *ad libitum*

Pinealectomy and sham operation were performed as described previously[2], and 10 d later a 2-mm semicircular wound was made in the right ear of each animal, including the controls After 72 h, colchicine (2 mg kg^{-1} body weight) was injected intraperitoneally All animals were killed 48 h later, always at 1030, to control for diurnal variations in pineal hormone

	Control	Sham	Melatonin	Pineal
Observations (*n*)	250 00	250 00	250 00	250 00
Mean	2 09	2 15	2 25	1 58
Median	2 00	2 24	2 27	1 73
Variance	0 60	0 46	0 57	0 53

interaction Daily weighing during the experiment showed no weight loss in any of the animals

For the sake of uniformity, each wound was divided into three areas Samples from the margins of each area were excised, fixed in neutral formalin, dehydrated and embedded in paraffin All samples were treated simultaneously to reduce the chance of artefact to a minimum Serial sections (7 μm) were cut, then stained with haematoxylin and eosin Each ear yielded 200 sections and all were mounted individually We selected 50 slides from each area of each wound randomly, and counted the cells in each high-power field ($\times 40$), the average number in each field was constant from animal to animal Obvious spindles and hyperchromatic nuclei with brush borders were considered to represent nuclei in mitosis and were included in the count

We took as response (*y*) the number of mitoses per high-power field in each slide Hence, the response was a discrete variable $0 \leqslant y \leqslant 20$ with 50 slides from each of five animals We had 250 mitotic values for each group The data showed that the responses did not follow normal distribution and the variances were heterogeneous Therefore, the square root transformation was used in the final analysis Table 1 summarises the transformed data The ANOVA (Table 2) clearly indicates that at

Table 2 ANOVA using square root transformation

Sources of variation	Degrees of freedom	Sum of square	Mean square	*F*
Between groups	3	68 1248	22 7083	42 3
Between animals within groups	16	11 4246	0 7140	1 33
Residual	980	526 0679	0 5368	
Total	999	605 6173		

least two group means are significantly different at the 1% level of significance To distinguish the pinealectomised groups from the others, a multiple comparison test was done The result indicated that control, sham-operated, and melatonin-treated animals form one group, with means not significantly different, whereas the pinealectomised group differed significantly from the others at the 5% level

The small number of mitoses in the healing wounds of pinealectomised mice is possibly the result of a direct hormonal effect on wound healing This observation is in contrast to the previous reports of gonadal hypertrophy and increased growth of malignant tumours after the removal of the pineal[2-4] The gonadal hypertrophy has been postulated to be the result of hyperplasia or of hypertrophy of individual cells of the target organ In the case of transplantable malignant tumours, hormonal influence has been proposed as one of the causes of increased growth and spread

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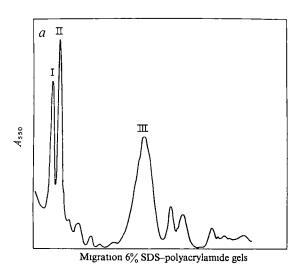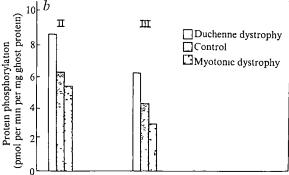
Membrane protein kinase alteration in Duchenne muscular dystrophy

DUCHENNE muscular dystrophy (DuD) is the severe form of heredo-familial muscular dystrophy inherited as a sex linked recessive trait The primary clinical manifestations are progressive muscle weakness usually starting in the pelvic girdle accompanied by hypertrophy in the calf musculature Significant cardiac abnormalities and mental retardation have also been reported The primary inherited metabolic defect is unknown[1] Elevated levels of muscle enzyme activities in the serum and their depletion in the muscle tissue have, however, long pointed to an abnormality in the plasma membrane as the probable site of the genetic defect

To explore potential membrane alterations in DuD we used a strategy which had been successful in another common form of hereditary familial myopathy, myotonic muscular dystrophy (MyD) Changes of denervation, atrophy, fatty infiltration, or fibrosis may make muscle membrane alterations impossible to interpret unless correlated with similar changes in other tissues So we used red cell membranes to explore the membrane abnormality in studies of MyD MyD red cell ghost phospholipids, polypeptides, and carbohydrates were similar to age and sex matched controls Endogenous protein phosphorylation, however, was decreased in fresh as well as frozen red blood cell membranes derived from patients with MyD[2,3]

Fig 1 *a*, 6% SDS-polyacrylamide gel scan of solubilised erythrocyte membrane protein No differences were demonstrated in scanning patterns from control, MyD, DuD or DuD carriers Molecular weights peak I, 210,000, II, 200,000, III, 90,000–100,000 *b*, Mean phosphorylation of major protein bands (II and III) of erythrocyte membrane measured under assay conditions (see text) Statistical data reported in Table 1

Migration 6% SDS–polyacrylamide gels

Other investigators have similarly demonstrated that clinically inapparent red cell alterations may accompany muscle disorders Layzer and coworkers have noted that the red cell contains a phosphofructokinase (PFK) composed of a muscle M-type subunit as well as a red blood cell or R-type subunit In the inborn error of metabolism affecting this enzyme the muscle M subunit is absent in red cell as well as the muscle and may be used as an index of the genetic defect[4] Morphological studies have similarly suggested red blood cell involvement in muscular dystrophy Using scanning electron microscopy, Matheson and Howland reported specific changes in the shape of RBCs derived from patients with DuD[5] In our own studies, we find no specificity Similar alterations were noted in red blood cells from patients with MyD, myotonic congenita, oculopharyngeal muscular dystrophy, homozygotes for DuD as well as carriers of DuD (S E Miller, A D R, and S H A, submitted for publication) Although our studies do not show that the morphological alterations are specific, they indicate clearly that the red blood cell membrane is involved in many muscular disorders

In this study, our initial intent was to determine whether the alterations in membrane protein kinase noted in MyD were specific for that disorder or were nonspecific alterations as noted in our morphological studies With the subsequent demonstration of a different level and pattern of protein phosphorylation in DuD, we were able to use this sensitive enzymatic reaction to monitor the membrane abnormality

Heparinised blood was obtained from DuD and MyD patients and matched with control heparinised blood Seven DuD patients from five families, three DuD carriers from three families, and seven MyD patients from four families were used Erythrocyte ghosts were prepared as previously described[3,6] All assays were performed on fresh ghosts without freezing or storage Incubations were performed as previously described and all assays were run in duplicate on 6% SDS-polyacrylamide gels Phosphorylation of the major erythrocyte protein peaks (II and III) (Fig 1) was linear for the incubation time of 1 min, and the protein concentration used $[\gamma\text{-}^{32}P]$-ATP was not limiting under these conditions[2,3]
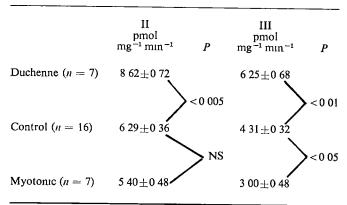
There were no reproducible differences in the protein polyacrylamide gel patterns of erythrocyte ghosts from DuD or DuD carriers from either MyD or controls Similarly phospholipid, fatty acid, and Ca^{2+}-ATPase activities were not significantly different[2]

The phosphorylation of protein bands II and III in DuD membranes was significantly increased compared with controls or MyD patients (Fig 1 and Table 1) No differences were noted between young male controls matched with DuD and the mixed adult control group matched with MyD and DuD carriers These data have been pooled in Table 1 Our previous demonstration of decreased phosphorylation of band III in MyD was confirmed in this series of experiments[3] Also no difference was again demonstrated in the phosphorylation of MyD band II compared with controls

DuD carrier band II phosphorylation was $8\,58\pm1\,03$ pmol per mg ghost protein per min This value is increased significantly compared with controls but is the same value as noted in DuD patients The similarity of the phosphorylation of DuD and DuD carrier band II phosphorylation suggests that one gene dose may be sufficient to reveal alterations in the endogenous protein kinase activity but may not be sufficient to produce clinical dystrophy Mild elevation of serum creatine phosphokinase activity in many carriers have been interpreted as reflecting partial leakage of the muscle membrane[7] Endogenous protein kinase activity may be closely related to the primary defect in DuD and represent a highly sensitive index of membrane alteration But further kinetic studies as well as specific substrate and enzymatic isolations will be necessary before definitive genetic interpretations may be made

These results demonstrate that abnormalities of protein

Table 1 Phosphorylation of erythrocyte protein bands

	II pmol mg^{-1} min^{-1}	P	III pmol mg^{-1} min^{-1}	P
Duchenne (*n* = 7)	8 62±0 72		6 25±0 68	
		<0 005		<0 01
Control (*n* = 16)	6 29±0 36		4 31±0 32	
		NS		<0 05
Myotonic (*n* = 7)	5 40±0 48		3 00±0 48	

Transfer of ^{32}P from [γ-^{32}P] –ATP to erythrocyte membrane protein band under assay conditions *n*, Number of patients or controls *P* is determined by Student's *t* test

phosphorylation are present in red cell membranes from patients with DuD and DuD carriers as well as from patients with MyD, and the specific pattern of alterations is distinctive for each disorder Our studies of MyD demonstrated that muscle membranes possessed a similar decrease in protein kinase activity as had been noted in red blood cell membranes[8] Our present results combined with previous morphological and biochemical data suggest that DuD like MyD is an inherited disorder of membranes with widespread tissue involvement

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Inhibitor(s) of prostaglandin synthesis in psoriatic plaque

IN 1926, Woronoff[1] described an area of blanched skin surrounding psoriatic lesions The mechanisms of this reaction were unknown but were thought to be a consequence of vasoconstriction In considering the probable explanation of the blanched area, we have noted that the blanched area is approximately the same width (1 cm) around the psoriatic lesion irrespective of the size of the lesion (suggesting that a substance diffuses out of the lesion), and the whitened area does not become red during ultraviolet light therapy Snyder and Eaglstein reported that the redness following exposure to ultraviolet light can be aborted by treatment with indomethacin, which is known to inhibit the synthesis of prostaglandins (PGs[2,3]) We have also found that (1) PGE$_2$ levels in whole skin removed from the blanched area are one-third of those in normal skin, (2) injection of PGE$_2$ into the blanched area produced redness, and (3) an inhibitor(s) of PG synthesis was
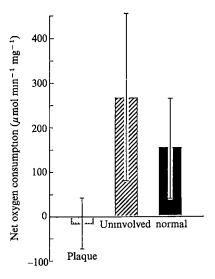
Fig. 1 Oxygen consumption of skin homogenates Aliquots of skin homogenate obtained from normal control patients and from plaque and uninvolved skin of psoriatic patients were treated as described in the text Oxygen consumption in the presence of dioxygenase and arachidonic acid alone was subtracted from the oxygen consumption in the presence of added homogenate and the difference is recorded as μmol O$_2$ min^{-1} mg^{-1} The difference in oxygen consumption between psoriatic plaque and uninvolved psoriatic skin is significant ($P < 0 001$) (Student's *t* test for small sample populations) whereas no statistically significant difference was noted between oxygen consumption in psoriatic uninvolved skin and normal control skin ($P > 0 1$)

present in this blanched area (our unpublished results) These observations suggested that a diffusable inhibitor of PG synthesis was present within the psoriatic lesion We now report the detection of an inhibitor(s) of PG synthesis in psoriatic plaque that is not present in extracts of uninvolved skin obtained from either psoriatic patients or normal volunteers

Punch biopsies (4 mm across) were obtained from psoriatic plaques (eleven) and uninvolved skin (nine) of psoriatic patients and from normal volunteers (eight) after injection of 1% xylocaine Skin specimens were homogenised in 100 mM Tris-HCl buffer, *p*H 8 5 Oxidation of arachidonic acid by sheep vesicular gland dioxygenase was carried out as described by Smith and Lands[4,5] Dioxygenase was prepared by homogenising aliquots of acetone powder of sheep vesicular gland (Upjohn) in 100 mM Tris-HCl buffer, *p*H 8 5 Enzyme preparations and skin homogenate were placed in the side of the electrode holder of an oxygen monitor Immediately thereafter, arachidonic acid (Sigma, 20 μM final concentration) was added to the mixture and the reaction rate (μmol O$_2$ min^{-1}) was determined by continuous measurement of oxygen uptake, using an oxygen electrode at 37 °C Protein was determined by the method of Lowry *et al*[6] using bovine serum albumin as the standard

In our assay system, normal human skin homogenates contained substances that increased oxygen consumption (Fig 1) The nature of this stimulation in oxygenation of arachidonic acid is unknown but is under study Oxygen consumption of psoriatic plaque homogenates was markedly depressed compared with that of normal appearing skin obtained from psoriatic patients or normal control patients (Fig 1) Oxygen consumption in the presence of psoriatic plaque extracts was less than that of dioxygenase and substrate alone (control), suggesting a direct interaction between an inhibitor(s) in the psoriatic plaque and dioxygenase, decreased oxygen consumption may also reflect an absence of nonspecific alternate reactions that consume oxygen Clinical observations coupled with other data (our unpublished results) indicate that the area surrounding the psoriatic lesion is deficient in PGE$_2$ during ultraviolet light therapy Our results suggest that the reduction of oxygen consumption in the presence of homogenate from

psoriatic lesions is the result of inhibition of sheep vesicular dioxygenase

The detection of an inhibitor(s) of PG synthesis explains the clinical phenomenon of the blanched area surrounding psoriatic plaques The relationship of this inhibition to the psoriatic process is unknown Studies are under way to define further the nature of the inhibitor(s), and preliminary experiments show that it is dialysable, not susceptible to ribonuclease or deoxyribonuclease, and not destroyed by proteases

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Role of macrophages in *in vitro* induction of T-helper cells

MACROPHAGES are of importance in the initiation of immune responses, both *in vitro*[1] and *in vivo*[1,2] Both immune responses involving B cells, such as antibody production[3-6], and those involving T cells, such as the lymphocyte transformation[7], the mixed lymphocyte reaction (MLR) (refs 8 and 9), and the induction of cytotoxic responses[10] require macrophages Various concepts of macrophage function in immune induction have been suggested The simplest concept proposed is that macrophages augment lymphocyte survival *in vitro*[11] Although this is one of their functions *in vitro*, it does not explain fully their role *in vitro* and especially *in vivo* In the induction of antibody responses, two functions were defined, the breakdown of antigens to the appropriate size[5] and the presentation of antigen to B cells in an optimally immunogenic manner[12] Because the T-cell reactions already studied, such as the MLR, involve ill-defined antigens, the surfaces of living cells, even less is known about the mechanisms of macrophage function in the stimulation of T cells than of B cells

Having recently described a system which enables unprimed T cells to be activated by soluble protein antigens and become helper cells[13], we investigated whether macrophages are involved in this reaction and attempt a definition of their role We report that macrophages were necessary, that there are genetic restrictions for effective T–macrophage interaction and that macrophages can be replaced by the supernatants of antigen-incubated macrophages of the same or an F_1 strain The genetic restrictions for effective T–macrophage interaction, however, did not apply if a particulate form of the antigen, keyhole limpet haemocyanin (KLH), conjugated to Sepharose beads (S–KLH), was used, indicating the existence of at least two different cellular pathways resulting in the generation of helper cells

Purified T cells were obtained from cortisone-resistant thymus cells (CRT) of CBA mice by the nylon wool technique[14], which removes adherent cells (macrophages and B cells) To generate helper cells, 15×10^6 adherent cell-depleted CRT were incubated with KLH for 4 d, together with syngeneic (CBA), allogeneic (BALB/c) or semi-allogeneic F_1 (CBA × BALB/c) macrophages obtained from peritoneal exudate In a second stage (cooperation), 10^5 of these cells were incubated together with 15×10^6 normal spleen cells and trinitrophenylated KLH (TNP–KLH) After 4 d, the anti-dinitrophenyl (DNP) or anti-TNP response was measured by counting plaque-forming
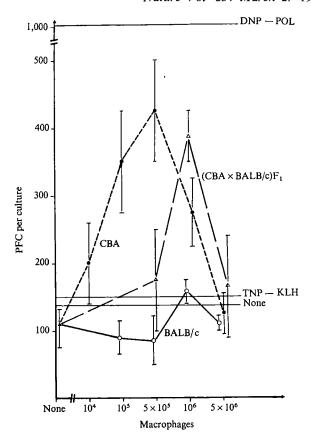
Fig 1 Requirement for syngeneic or semi-allogeneic macrophages in the induction of T-helper cells PE from CBA, BALB/c and (CBA × BALB/c)F_1 mice were used as a source of macrophages To stimulate PE, mice had been injected with 1 ml 10% sterile Proteose–Peptone broth (Oxoid Code L46) 3 or 4 d previously Unstimulated PE were just as effective as stimulated PE The PE were washed in saline and treated with a sheep anti-T serum (dilution 1 4) and spleen cell adsorbed guineapig complement (1 8) for 45 min at 37 °C After washing, macrophages were incubated in graded numbers with 15×10^6 nylon wool purified CRT cells from CBA mice and 0 1 μg KLH in 1 ml HEPES-buffered Eagle's MEM containing 10% foetal calf serum in small (1 cm) Marbrook inserts for 4 d at 37 °C in an atmosphere of 10% CO_2 in air The outside compartment contained 25 ml of bicarbonate-buffered MEM with 5% normal calf serum As a control, no macrophages were added to the purified CRT After 4 d incubation the cells were collected, washed and 10^5 viable cells added to 15×10^6 CBA spleen cells containing 0 1 μg ml^{-1} TNP–KLH in Marbrook flasks (as above) and incubated for 4 more d The effect of macrophages on the generation of helper cells was measured by the cooperation between the carrier-activated T cells and the hapten-activated B cells expressed by the IgM plaque-forming ability of B cells against DNP or TNP-coated SRBC In our cultures, unprimed B cells do not make IgG

cells (PFC) against TNP- or DNP-coated sheep red blood cells (DNP-SRBC) thereby giving a measure of the helper activity generated in the first culture

Figure 1 shows that no helper cells were generated in the absence of macrophages, as indicated by the number of PFC which fell in the background range With the addition of increasing numbers of syngeneic macrophages to adherent cell-depleted CRT the helper activity increased as shown by the number of PFC generated Addition of 5×10^5 macrophages gave the highest number of PFC, indicating optimal helper activity A response was not seen when macrophages from BALB/c mice were incubated with CBA T cells, but was seen when they were incubated with BALB/c T cells (P E , and M F , unpublished), indicating that either T cells and macrophages must be histocompatible or that allogeneic macrophages exert an inhibitory effect on the helper cell induction The latter possibility has been excluded, since helper cells are obtained, if a mixture of CBA and BALB/c macrophages are incubated with

Table 1 Capacity of supernatant from syngeneic or semi-allogeneic macrophages (incubated with KLH for 4 d) to induce helper cells

First culture			Second culture		
Helper cell induction			Cooperation		
T cells (Macrophage-depleted spleen)	Antigen	Macrophage or supernatant	Helper cells transferred 10^5	Challenge	Anti-DNP-response (PFC ± s.e.)*
+	KLH	Nil	+	TNP–KLH	65 ± 35
+	KLH	5×10^5 macrophage	+	TNP–KLH	274 ± 39‡
+	Nil	CBA S/N₄ KLH(5%)	+	TNP–KLH	284 ± 34§
+	KLH	CBA S/N₄ (40%)	+	TNP–KLH	70 ± 5
+	KLH	CBA S/N₄ (20%)	+	TNP–KLH	93 ± 52
+	KLH	CBA S/N₄ (1%)	+	TNP–KLH	62 ± 17
+	Nil	BALB/c S/N₄ KLH(5%)	+	TNP–KLH	68 ± 28
+	Nil	F₁ S/N₄ KLH(10%)	+	TNP–KLH	293 ± 48‖
+	Nil	F₁ S/N₄ KLH(1%)	+	TNP–KLH	167 ± 40
—	—	—	—	TNP–KLH	123 ± 38†
—	—	—	—	DNP–POL	647 ± 142
—	—	—	—	Nil	111 ± 41

Spleen cells were used for the induction of T-helper cells. The adherent and phagocytic cells were removed by incubating 200×10^6 spleen cells (in 10ml of HEPES-buffered MEM containing 10% foetal calf serum) with 0.1 g carbonyl–iron in a 25 ml glass beaker for 1 h at 37 °C, by keeping a strong magnet on the bottom of the beaker while pouring the cells carefully into another beaker. The magnet treatment was repeated once, the cells washed and cultured in small (1 cm) Marbrook vessels with or without antigen (KLH, 0.1 μg ml⁻¹) and macrophage or macrophage supernatant. To obtain pure macrophage supernatant, 5×10^6 CBA, BALB/c or F₁ PE were incubated in the wells of a Linbro culture plate (No. FB–16–24 TC, Biocult, Glasgow) for 2 h at 37 °C, the non-adherent cells removed by extensive washings with HEPES-buffered MEM. Residual T and B cells were removed by treating with undiluted anti-T and anti-B serum and complement for 45 min at 37 °C and washing twice with MEM. The remaining cells (macrophage) were 99.7% phagocytic as judged by uptake of polystyrene beads. The macrophages were then incubated with 25 μg KLH (S/N₄ KLH) or no KLH (S/N₄) in 1 ml of bicarbonate-buffered MEM containing 10% foetal calf serum for 4 d at 37 °C. The supernatant (S/N) was then removed, spun for 15 min at 20,000g and frozen until use (1 ml supernatant was taken as 100%). Before use the supernatant was filtered through 0.45 μm Millipore filter. For the cooperative response, 10^5 viable cells from the first culture were incubated with 15×10^6 normal spleen cells containing 0.1 μg ml⁻¹ TNP–KLH in Marbrook flasks and incubated for 4 d at 37 °C. Dinitrophenylated polymeric flagellin (1.0 μg ml⁻¹) was used as a control. The anti-DNP response was measured by the IgM plaque-forming ability of the B cells against DNP-coated SRBC.
 * Antibody-forming cells per culture ± s.e. Results are pooled from three experiments.
 † Used as control for calculation of statistics by Student's *t* test. Levels of significance: ‡ $P < 0.05$; §$P < 0.01$; ‖$P < 0.02$.

CBA T cells (P. E., and M. F., unpublished). F₁ macrophages were able to generate helper cells, although more semi-allogeneic macrophages were necessary to give a response. But this response was always less than with syngeneic macrophages. The need for syngeneic or semi-allogeneic cells for a successful macrophage–lymphocyte interaction was previously described in guineapigs[15], but was not found in mice[16,17].

In a second set of experiments it was ascertained that macrophages incubated in the presence of antigen, for example, KLH, can release factor(s) able to induce specific helper cells to KLH. Table 1 shows that supernatant taken from purified macrophages, which had been incubated with KLH for 4 d, adequately replaced macrophages and antigen when tested in the helper system. Thus in this system no direct contact between macrophages and T lymphocyte is necessary. Moreover, in accordance with the findings described above, the supernatant raised from allogeneic (BALB/c) macrophages were ineffective,

whereas that derived from F₁ macrophages activated helper cells; and that obtained from syngeneic macrophages incubated in the presence of KLH had no effect, even in high doses.

By contrast, the latter supernatant was effective if used with a particulate antigen, KLH bound to agarose (Table 2), suggesting the existence of different mechanisms of helper cell activation by soluble and particulate antigens. Moreover, the supernatant from allogeneic macrophages had the capacity to induce helper cells, if tested in the presence of KLH bound to agarose (P. E., and M. F., unpublished). The chemical nature and biological significance of a 'specific' factor(s) which replaces antigen and macrophages in helper cell induction, and a nonspecific factor(s) which replaces macrophages, but only in the presence of particulate antigens, remains to be investigated. There are several reports of such nonspecific replacing factors[18-21], but factors derived from macrophages, capable of replacing both macrophages and antigen have not been

Table 2 Capacity of supernatant from macrophages (incubated for 4 d with KLH bound to sepharose) to induce helper cells

First culture			Second culture		
Helper cell induction			Cooperation		
T cells (macrophage-depleted spleen)	Antigen*	Macrophage or supernatant	Helper cells transferred (10^5)	Challenge	Anti-DNP-response (PFC ± s.e.)
+	S–KLH	Nil	+	TNP–KLH	83 ± 27†
+	S–KLH	5×10^5 macrophage	+	TNP–KLH	277 ± 68‡
+	S–KLH	S/N₄ KLH(5%)	+	TNP–KLH	265 ± 34‡
+	S–KLH	S/N₄ (5%)	+	TNP–KLH	285 ± 43‡
—	—	—	—	TNP–KLH	77 ± 19
—	—	—	—	DNP–POL	634 ± 161
—	—	—	—	Nil	73 ± 23

* KLH was bound to Sepharose 2B using cyanogen bromide. Sepharose beads (10^1 per culture) were used. TNP–KLH was used at 0.1 μg ml⁻¹, DNP–POL at 1.0 μg ml⁻¹. Results are pooled from three experiments.
 †Used as control for calculation of statistics by Student's *t* test. ‡ Level of significance: $P < 0.02$.

described previously. The analysis of the genetic requirements for T lymphocyte–macrophage interaction should also be of interest since other forms of cell interaction have recently been suggested to have genetic (H-2-lined) restrictions, for example, T–B cooperation[22] and the killing of virus-infected cells[23].

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Centromeric asymmetry and induction of translocations and sister chromatid exchanges in mouse chromosomes

THE mechanisms involved in chromosome rejoining are not understood. We showed that chromosome rejoining in Robertsonian centric fusion can be studied cytologically[1] with a staining technique based on the quenching by 5-bromodeoxyuridine (BrdU) of the fluorescence of the dye 33258 Hoechst[2]. When mouse cells were grown for one generation in the presence of BrdU and stained with 33258 Hoechst, there was a brightly fluorescent spot appearing over half of the centromeric region in every autosome[3]. (This asymmetry presumably reflects the unequal distribution of thymidine (45% compared with 22%) between the two chains of mouse satellite DNA[4], the fluorescent spot representing the old thymidine-rich chain of satellite DNA.) The arrangement of fluorescent spots in metacentric chromosomes resulting from Robertsonian fusion suggested that the thymidine-rich chain of satellite DNA in the centromeric region is associated with the same DNA chain (in terms of polarity) in every mouse autosome[1]. We have used the centromeric asymmetry in mouse chromosomes as a marker for DNA polarity in studies on radiation and drug-induced chromosomal translocation and sister chromatid exchange (SCE).

Mouse cells from two permanent lines (RAG and A9) were grown in Eagle's medium with 10% foetal calf serum. For radiation studies, cells growing in Falcon tissue culture flasks were exposed to X rays (350 rad, 190 kV). Thirty minutes before irradiation colcemid ($0.45\ \mu g\ ml^{-1}$) was added to the cultures, and 3 h after irradiation BrdU ($10^{-5}M$)
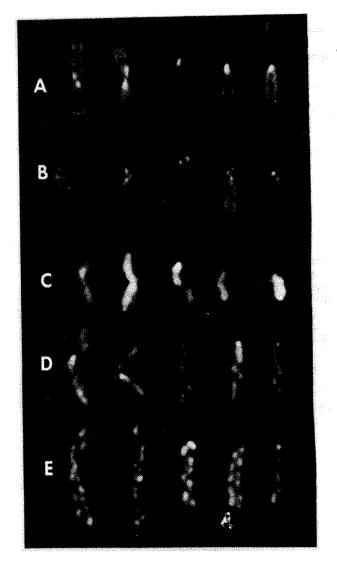
Fig. 1 Fluorescent staining of X-ray-induced dicentric chromosomes. RAG cells were grown for one cycle of replication in BrdU before fixation and staining with 33258 Hoechst. *a* and *b*, telocentric and metacentric chromosomes from unirradiated cells. *c* and *d*, Telo-dicentric and telo-meta-dicentric chromosomes from irradiated cells, showing the fluorescent spots on the opposite sides of the chromosomes. *e* and *f*, The same as (*c*) and (*d*), but with the fluorescent spots on the same sides of the chromosomes. *g*, Chromosomes from irradiated cells, showing SCE within the centromeric region.

or a mixture of BrdU ($10^{-5}M$), fluorodeoxyuridine ($2 \times 10^{-5}M$) and uridine ($3 \times 10^{-4}M$) was added. The cells were fixed 24–25 h after irradiation. For studies with drugs, cells were grown in medium containing mitomycin C (MC) ($0.5\ \mu g\ ml^{-1}$) and BrdU ($10^{-5}M$) for either 22–24 h or 48 h and then fixed. In one experiment, cells were grown for 18 h in BrdU alone and then for 22–24 h in BrdU and MC together. The techniques for chromosome preparation and analysis have been described previously[2,3].

The relationship between DNA polarity and chromosomal rejoining in X-ray induced translocations was examined cytologically, using the fluorometrically detectable centromeric asymmetry (Fig. 1*a* and *b*) as a cytological marker for the chain of DNA with a given polarity. In non-irradiated RAG cells, end-to-end translocations were not seen. But, one generation after irradiation, (distal) end to (distal) end translocations appeared in more than 25% of metaphases. Both telo-dicentric and telo-meta-dicentric chromosomes (resulting respectively from translocation between two telocentric or between telocentric and metacentric chromosomes) were observed (Fig. 1*c–f*). (Isodicentric chromosomes

resulting from iso-chromatid breakage and proximal union should not be present until the second metaphase after irradiation[5].) In every telocentric unit, a brightly fluorescent spot was associated with half of the centromeric region. In every metacentric unit two bright centromeric spots were arranged contralaterally. In the telo-dicentric chromosomes, the bright spots in the two centromeric regions could be arranged *a priori* either on the same side (relative to the long axis) or on opposite sides of the chromosome. In more than 95% of the telo-dicentric chromosomes, however, the bright spots were located on opposite sides of the chromosomes (Table 1). Similar results were obtained with the telo-meta-dicentric chromosomes. Since the spots presumably were associated with the DNA strand of the same polarity in every autosome, these results suggest that the orientation of the joined chromosomes in end to end translocations is such that DNA polarity is maintained from one centromere through the point of joining to the other centromere. The small proportion of telo-dicentric chromosomes in which the two bright spots were located on the same side of the chromosome (fewer than 5%) could be the result of X-ray-induced SCE at some point between the centromeres.

In the analysis of non-irradiated A9 cells, a stable chromosome aberration (appearing in more than 90% of the cells) was observed. The novel chromosome appears to have arisen from end-to-centromere translocations involving three chromosomes. After one cycle of replication in BrdU, the chromosome had three brightly fluorescent spots, all on the same side (Fig. 2). Assuming that this chromo-

Fig. 2 Fluorescent staining of a spontaneous chromosomal aberration. A9 cells were grown for one cycle of replication in BrdU before fixation and staining with 33258 Hoechst. Each chromosome is from a different cell.

some arose by translocation, these results suggest that the three chromosomes involved in the translocation were oriented such that the polarity of the DNA was maintained through the three regions of satellite DNA. (The stability of this translocation suggests that two of the three centromeres in this chromosome are either non-functional or absent.)

Our results suggest that DNA polarity is involved in determining the orientation of chromosomes during end-to-end and end-to-centromere translocations. This is consistent with the results of previous studies on other types of chromosomal reorganisation[1,5,6].

We have also used centromeric asymmetry to study SCE. The effects of X rays and MC, both previously shown to increase the frequency of SCE[7-9], were investigated. In RAG cells grown for one generation in BrdU, SCE within the centromeric region (appearing as a split in the fluorescent spot) occurred with a very low frequency (0.05 per metaphase). At the first division after X irradiation (Fig. 1g) the frequency of SCE within the centromeric region increased to 0.3 per metaphase. After one cycle of replication in MC plus BrdU (Fig. 3b), the frequency increased to 11.2 per metaphase.

In contrast to the centromeric region, SCE in the non-centromeric region was not observed at all after one cycle of replication in MC plus BrdU (Fig. 3b). After two cycles of replication in MC plus BrdU, however, the effect of MC

Table 1 Arrangement of fluorescent spots in the centromeric region of X-ray-induced dicentric chromosomes

Arrangement of spots	Telo-dicentric	Telo-meta-dicentric*	Total
Opposite side	730	92	822
Same side	32	10	42

RAG cells were irradiated and grown for one generation in BrdU and stained as described in the text. The data were collected from 292 metaphases, all of which contained at least one dicentric chromosome.
* For these chromosomes, the arrangement refers only to the spots associated with the two centromeric regions on either side of the point of joining.
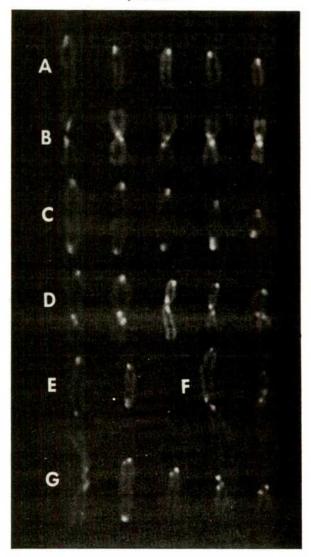
was obvious (Fig. 3e). RAG cells grown for two cycles in BrdU without MC had on the average 12 SCE per metaphase; RAG cells grown for two cycles in BrdU with MC had more than 200 SCE per metaphase.

The difference between the appearance of MC-induced SCE in the centromeric and non-centromeric regions is not caused by a difference in the effect of MC in the two regions. If cells were grown for one generation in BrdU and then for a second generation in MC plus BrdU, there was a marked increase in the frequency of SCE (to 92 per

Fig. 3 Fluorescent staining of MC induced SCE. RAG cells were grown for different times in MC plus BrdU before fixation and staining with 33258 Hoechst. *a*, Cells grown for one generation in BrdU without MC. *b*, Cells grown for one generation in MC plus BrdU, showing SCE only in the centromeric region. *c*, Cells grown for two generations in BrdU without MC. *d*, Cells grown for two generations in BrdU, with MC present only during the second generation. *e*, Cells grown for two generations in MC plus BrdU.

metaphase) in the non-centromeric as well as the centro-meric region (Fig. 3d). Thus MC-induced SCE in the non-centromeric region can be observed in the first division following MC treatment, but only if BrdU is added one generation before MC. The failure to observe MC-induced SCE in the non-centromeric region until the second meta-phase when MC and BrdU are added together is in agree-ment with autoradiographic results that suggested that SCE involves exchanges of double stranded DNA molecules rather than exchanges of single polynucleotide strands[10]. The ability to detect SCE in the centromeric region at the first metaphase when MC and BrdU are added together is due presumably to the asymmetric distribution of thymi-dine in the two strands of mouse satellite DNA. This natural asymmetry thus provides a system in which SCE can be detected within the first generation after labelling, which could facilitate certain types of studies on chromosome breakage and rejoining.

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Fig. 1 *a*, Time course of hybridisation reactions using RNA from nuclei ($\triangle$) and polysomes ($\bullet$) of PY AL/N cells. Data are corrected for DNA self-reassociation as determined from controls which contained DNA alone[1]. *b*, Double-reciprocal plots of the data in (*a*). The procedures for isolating and characterising non-repetitive DNA labelled with thymidine-2-[14]C have been published[1]. To obtain RNA, cells were removed from the bottles with 0.05% trypsin+0.02% EDTA, washed twice in serum-free medium and resuspended at 8×10^6 cells ml^{-1} at 0 °C in 10 mM Tris, pH 8.4, containing 0.14 M NaCl and 1.5 mM $MgCl_2$. Nuclear and cytoplasmic fractions were prepared using 0.05% Triton X-100[2]. Nuclear RNA was obtained by hot phenol extraction[1]. Polysomes were purified from the supernatant by centrifugation through discontinuous sucrose gradients[7]. Polysomal RNA was isolated by phenol–chloroform extraction[8]. Nuclear and polysomal RNA preparation were treated with DNase and passed over columns of Sephadex G-100 (ref. 1). Hybridisation reactions containing the appropriate RNA (12 mg ml^{-1}) and labelled non-repetitive DNA (12 µg ml^{-1}) were carried out as previously described[1].

Non-repetitive DNA transcripts in nuclei and polysomes of polyoma-transformed and non-transformed mouse cells

WE have determined the extent to which non-repetitive DNA is represented in nuclear and polysomal RNA of polyoma-transformed and non-transformed mouse cells in culture. Our RNA–DNA saturation-hybridisation experiments have shown that in transformed cells, polysomes contain only 10–20% of the informational complexity present in nuclear RNA. Similar results were obtained with RNA from confluent cultures of non-transformed cells. In subconfluent cultures of non-trans-formed cells, however, polysomes contained about 40% of the sequence diversity present in nuclear RNA. Further experiments, with appropriate RNA mixtures, suggest that there are also qualitative differences in polysomal RNA between transformed and non-transformed cells, as well as between confluent and subconfluent cultures of non-transformed cells.

The results of experiments using RNA from the nuclei of PY AL/N cells and from polysomes prepared by centrifugation through discontinuous sucrose gradients are shown in Fig. 1. On the assumption that only one strand of DNA is transcribed the saturation values were taken as twice the intercept values of the double-reciprocal plots. The value obtained for nuclear RNA (30%) is the same as that reported earlier using total RNA from these cells[1]. The average saturation value for polysomal RNA was 6.4% (four experiments, range 5.2–8.0%).

Two experiments were carried out to determine the degree to which the polysomal RNA might be contaminated with RNA released from nuclei that were lysed or damaged during cell fractionation. First, DNA was labelled with thymidine-2-[14]C, and nuclei and polysomes were isolated as usual. Approximately 99.5% of the acid-insoluble counts remained in the nuclei; only 0.5% was found in the polysomes. The second experiment was based on reports[2,3], that when cells are labelled with uridine, no counts appear for 10–15 min in either polysomal messenger RNA or cytoplasmic ribosomal RNA. In cells labelled with uridine-2-[14]C for 10 min and fractionated as usual, the distribution of radioactivity between nuclei and polysomes was identical to that observed when DNA was labelled. When these nuclei were treated with DNase, lysed with SLS and centrifuged as in the preparation of polysomes, 14% of the acid-insoluble counts was recovered in the pellet. If this is a reasonable estimate of the maximum amount of nuclear RNA that could contaminate the polysomes, the 0.5% found above suggests an upper limit of contamination of about 3–4%.

A second set of experiments was carried out using more stringent criteria to define polysomal RNA[4,5]. Polysomes were centrifuged through linear sucrose gradients and the material sedimenting at > 100S was collected. After treatment with EDTA, this fraction was again centrifuged through a sucrose gradient and the portion sedimenting at < 80S was collected and the RNA was extracted. The results of hybridisation experiments using such RNA are presented in Fig. 2. The saturation values for two experiments (3 and 4%) are in fairly good agreement with those obtained by Bishop *et al.*[6] using the poly(A)-containing RNA from the cytoplasm of HeLa cells.

In the case of non-transformed cells it was necessary to collect from many bottles to obtain adequate quantities of RNA. Therefore, polysomes were prepared only by the procedure involving centrifugation through discontinuous sucrose gradients. In the experiments with transformed cells, polysomal RNA isolated by this method gave the highest

Table 1 Saturation values of RNA–non-repetitive DNA hybridisation experiments with RNA from non-transformed AL/N cells

	Nuclear RNA Average	(Range)	No of experiments	Polysomal RNA Average	(Range)	No of experiments
Growth state						
Confluent	20	—	1	5 7	(5 0–6 4)	3
Subconfluent	19	—	1	8 6	(8 4–8 8)	3

Each experiment used the appropriate RNA (12 mg ml^{-1}) and non-repetitive DNA (12 µg ml^{-1}) Hybridisation reactions were carried out as described in Fig 1 Assuming that only one strand of DNA is transcribed, the intercepts of double-reciprocal plots were doubled to obtain the tabulated values

saturation values, consequently the measurements with non-transformed cell RNA are likely to be maximum estimates of the RNA sequence diversity in polysomes

The saturation values obtained with nuclear and polysomal RNA of nontransformed cells are summarised in Table 1 As with transformed cells, the saturation values obtained with nuclear RNA from confluent and subconfluent cells agree with those previously obtained using total RNA from these growth states[1] The values for polysomal RNA from confluent (5 7%) and subconfluent (8 6%) cells suggest that more non-repetitive DNA is represented in the latter Experiments using a mixture of RNA from the two growth states gave saturation values of 10 4% and 11 2%, implying qualitative as well as quantitative differences with respect to the sequence diversity of polysomal RNA in confluent and subconfluent cells

Qualitative differences between the polysomal RNA species in transformed cells and subconfluent non-transformed cells are also suggested, since experiments with RNA mixtures yielded saturation values of 9 2% and 11 6%

To determine whether the hybrids contained only non-repetitive DNA, the DNA was isolated from the hybrids and its reassociation behaviour was examined in the presence of an excess of unlabelled, unfractionated mouse DNA As Figure 3 shows, except for 5% which bound to hydroxylapatite immediately after denaturation, the DNA from the hybrids reassociated as expected for nonrepetitive sequences The labelled DNA used in these experiments contained some sequences resulting from DNA self-reassociation, as well as sequences from RNA–DNA hybrids Even assuming that only 50% of the hybridised DNA originated from RNA–DNA duplexes

Fig 3 Reassociation of DNA isolated from hybrids in two experiments Hybridised DNA was isolated from hydroxylapatite and the RNA was removed by alkaline hydrolysis The DNA was dialysed into distilled water Sheared, unlabelled and unfractionated mouse embryo DNA was added to the sample to a concentration of 1 0 mg ml^{-1} and the ionic strength was adjusted to 0 12 M phosphate buffer The sample was denatured and incubated at 60 °C Samples were removed periodically and assayed on hydroxylapatite The reassociation of the unlabelled DNA was determined by the A_{260} (O), and that of the labelled DNA on the basis of its radioactivity (● and △)

Fig. 2 *a*, Time course of hybridisation experiments using the >100S PY AL/N RNA that sediments at <80S after EDTA treatment *b*, Double-reciprocal plot of the data in (*a*) Hybridisation conditions were similar to those in Fig 1 Cells were lysed, nuclei removed, and polysomes isolated as described in Fig 1 Polysomes suspended in buffer (10 mM Tris, pH 7 4, containing 10 mM NaCl and 8 mM MgCl$_2$) supplemented with polyvinyl sulphate (50 µg ml^{-1}), hereafter referred to as B+PVS, were layered over 30 ml, linear 15–30% (w/v) sucrose gradients (sucrose in B+PVS) built over a 2 0 ml cushion of 60% sucrose After centrifugation in a SW27 rotor at 25,000 r p m and 4 °C for 3 h, the material sedimenting at >100S was pooled and diluted 1 2 with B+PVS, and the polysomes were pelleted by centrifugation overnight in a type 60 Ti rotor at 55,000 r p m and 4 °C Polysomes were resuspended in B+PVS and the solution was made 25 mM EDTA The treated polysomes were layered over 15–30% linear gradients and centrifuged as before The material sedimenting at <80S was collected, MgCl$_2$ was added to 5 mM, and the polysomes were precipitated by adding ethanol to 50% and storing overnight at −10 °C The polysomes were collected by centrifugation at 25,000*g* for 15 min and resuspended in 10 mM Tris, pH 7 4, containing 0 1 M NaCl, 1 mM EDTA and 0 5% SLS RNA was isolated by phenol–chloroform extraction[8]

and that all the material which initially bound to hydroxylapatite occurred in this fraction, the results still indicate that at least 90% of the DNA which hybridised with RNA was comprised of non-repetitive sequences

To assess the precision of base pairing in the hybrids formed, their thermal stability was determined Samples were bound to columns of hydroxylapatite and eluted as a function of temperature at 5 °C intervals The thermal dissociation patterns resembled those obtained for total RNA–DNA hybrids[1], the midpoints of the transitions were 79–80 °C We concluded that no extensive mismatching of base pairs occurred in these hybrids

The results of these experiments can be summarised as follows First, although the saturation values obtained in RNA–DNA hybridisation reactions show a dependence on the method used for polysome isolation, only a fraction of the non-repetitive DNA transcripts found in the nucleus of either transformed or non-transformed cells are also found in the polysomes Second, confluent and subconfluent cultures of non-transformed cells are identical in the amount of non-repetitive DNA represented in nuclear RNA On the other hand, both quantitative and qualitative differences exist in the non-repetitive transcripts in the polysomes of cells in these growth states Third, the nuclear RNA of the virus-transformed cells contains a more diverse population of non-repetitive DNA transcripts than does the nucleus of the non-transformed cells In contrast, non-repetitive DNA is represented equally in the polysomes of transformed and

non-transformed cells Qualitative differences exist, however, in the polysomal sequences of these two types of cell

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Different susceptibility of DNA and RNA to cleavage by metal ions

METAL ions are required in virtually every phase of genetic information transfer, and they are generally essential components of biochemical processes involving DNA and RNA Under certain conditions, however, metal ions can have deleterious effects, and one of these, the degradation of polyribonucleotides, is not frequently considered by those who deal with nucleic acids in the presence of metals No such degradation has been demonstrated with DNA, although it is known[1] that DNA, like RNA, is subject to thermal degradation, and therefore can be expected to undergo some degradation also in the presence of metal ions It is important to understand the relative susceptibility of polydeoxynucleotides and polyribonucleotides to degradation by metal ions to answer the following questions To what extent is DNA, compared with RNA, vulnerable to metal ion degradation during biochemical experiments? What are the implications for metal ion toxicology? Is the difference in susceptibility to metal hydrolysis sufficient to make possible a quantitative separation of degraded RNA from undegraded DNA in a DNA–RNA mixture? Finally, does this difference in susceptibility reflect differences in the intrinsic stabilities of DNA and RNA, and perhaps throw light on the reason for the evolutionary selection of DNA, rather than RNA, as the favoured bearer of the primary genetic information? These questions can be answered by a comparison of DNA and polyribonucleotide degradation by metal ions using a method of detection sensitive enough to respond to the first break in the macromolecule

Polyribonucleotides are readily degraded by heating in the presence of various metal ions[2][3] Zinc(II) ion[3] is a very effective degrading agent, and the characteristics and mechanism of the zinc reaction have been thoroughly investigated[3-5] The zinc degradation of poly(rA) is typical of that of polyribonucleotides[2] For these reasons we have chosen to compare the degradation, in the presence and absence of zinc(II) ion, of poly(rA) with that of calf thymus DNA The non-degradability of DNA by metal ions has been suspected[2][6,7] on the basis of assays which could, however, detect only very extensive cleavage By following the zinc cleavage reaction by sedimentation velocity measurements, in conditions of strand separation, we can detect even one break per strand The reactions were carried out at pH 7 0, low ionic strength, with and without 2 Zn per nucleotide residue, and, for comparison of single and double stranded DNA, at each of two temperatures 50 °C, which is below the melting range of the DNA under these conditions, and 80 °C, which is above it[7] (Poly(rA) is single stranded at both temperatures[8,9])
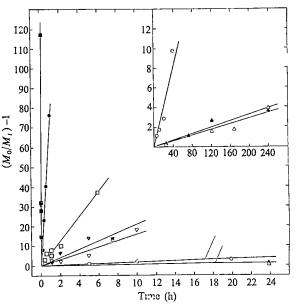
Fig 1 Time course of cleavage of calf thymus DNA and poly(rA) at 50 °C and 80 °C, pH 7, with and without 2 Zn per nucleotide residue, as measured by reduction of the weight average, single-strand molecular weight during times t from the initial value M_0 to values M_t DNA 50 °C, (▲) with Zn, (△) without, 80 °C, (▼) with Zn, (▽) without Poly(rA) 50 °C, (●) with Zn, (○) without, 80 °C, (■) with Zn, (□) without Calf thymus DNA $(M_0 = 1\ 2 \times 10^6)$ was from Worthington Biochemicals, the stock solution was prepared as before[11] Poly(rA) $(M_0 = 0\ 41 \times 10^6)$ was from Miles Laboratories Reaction mixtures contained 0 10 mM nucleotide residue and 5 mM $NaNO_3$, and zinc ion was introduced as $Zn(NO_3)_2$, pH was initially adjusted to 7 0 by addition of NaOH For sedimentation measurements, zinc was removed from DNA solutions by batch exchange on Dowex-50(Na), and then to separate the strands[12] NaOH was added to 0 1 M and NaCl to 1 M, poly(rA) solutions were made 0 01 M in sodium EDTA buffer, pH 7 0 and 0 1 M in NaCl[3] (poly(rA) remains single stranded under such conditions[8,9]) Weight average sedimentation coefficients were determined at 20 °C (Beckman Model E ultracentrifuge, ultraviolet absorption optics) from the movement of the half-maximal position of the integral boundary pattern Weight average molecular weights were calculated for DNA, using the correlation of Studier[12] for the alkaline medium, $S_{20,w} = 0\ 0528\ (M^{0\ 40})$, for poly(rA), from the correlation of Fresco and Doty[13] for neutral 0 15 M NaCl, $S_{20,w} = 0\ 024\ (M^{0\ 45})$ (S in Svedbergs)

The sedimentation results, in terms of the calculated weight average, single-strand molecular weights, are presented in Fig 1 It is immediately obvious from Fig 1 that a large difference in susceptibility to zinc cleavage exists between single or double stranded DNA, and poly(rA), so that very extensive zinc depolymerisation of poly(rA) occurs during a time in which DNA sustains negligible damage Some cleavage of the DNA—both with and without zinc—was eventually detected, even at the lower temperature (inset of Fig 1), but the effect of zinc on DNA cleavage was insignificant, both at 50 and 80 °C, compared with its effect on the cleavage of poly(rA)

All the plots of Fig 1 fit straight lines reasonably well Table 1 provides quantitative comparisons of the slopes of these plots, which represent rates of depolymerisation on a single strand basis The Table also lists times required for reduction of the molecular weight to given fractions of the initial value Table 1 shows that there was no significant added effect of zinc on the cleavage of DNA, either above or below the melting temperature, while there was a 200–300-fold increase in the rate of depolymerisation in the case of poly(rA) The rate for poly(rA) was 4,000 times greater than the rate for DNA, in the presence of zinc, when the DNA was double stranded (at 50 °C) When the DNA was single stranded (at 80 °C), poly(rA) was still degraded 1,000 times more rapidly than DNA In the absence of zinc the degradation of poly(rA) was only 14 times faster at 50 °C and four times faster at 80 °C

These results indicate that cleavage induced directly by metal

Table 1 Rate of reduction of the weight average, single-strand molecular weight of calf thymus DNA, and of poly(rA), at 50 °C and 80 °C, pH 7 0, with and without 2 Zn per nucleotide residue

	Slope (h⁻¹) of M_0/M_t against t*		Approximate time (h) for reduction of M_0†			
			to 1/2 M_0		to 1/10 M_0	
DNA	+Zn	−Zn	+Zn	−Zn	+Zn	−Zn
50 °C	0 016±0 002	0 014±0 003	62	71	560	640
80 °C	2 1±0 6	1 6±0 2	0 48	0 62	4 3	5 6
Poly(rA)						
50 °C	72±4	0 20±0 04	0 014	5 0	0 12	45
80 °C	1,400±400	6 2±0 6	0 00064	0 16	0 0062	1 4

* Evaluated from the plots of Fig 1 (the mean deviation for fit to the experimental points are appended) M_0, initial weight average molecular weight, M_t, values at times t
† Calculated from the slope of M_0/M_t against t, $M_0/M_t = 1 + (\text{slope})t$

ions is generally unlikely during *in vitro* manipulations of DNA, although it must be taken into account with polyribonucleotides Figure 1 and Table 1 can serve as a guide to the amount of damage to be expected under various conditions

The great difference in the susceptibility of polyribonucleotides, compared with polydeoxynucleotides, to zinc cleavage makes possible the quantitative removal of RNA from a DNA–RNA mixture This becomes evident when one calculates from the data of Table 1 that, at 50° C with zinc, about 5,000 breaks occur in a poly(rA) strand for one break in a DNA strand Selective zinc degradation of RNA has already been utilised[10] in connection with chromatin reconstitution studies, our results indicate that the DNA components remain intact during such degradation

Our results suggest also that DNA should be much less susceptible than RNA to deleterious action by metal ions *in vivo* Thus the question arises whether these effects may be part of the reason for the predominant evolutionary selection of DNA, instead of RNA, as the bearer of the primary genetic information

Obviously, any attempt to answer this question, as any question involving evolutionary hypotheses, must be speculative It has been suggested[1] that the relatively small difference in thermal stability of DNA and RNA can account for the choice of the DNA duplex as the repository of genetic information But this difference in thermal stability is only about one three-hundredth the difference in stability to metal ions Moreover, metal ion degradation of polyribonucleotides proceeds[5] through a mechanism that involves the 2′ OH group, the presence of which in RNA and absence in DNA constitutes the only primary structural difference between the two types of nucleic acid For both of these reasons, if stability of the primary genetic material is a criterion of its evolutionary selection, susceptibility towards metal ions seems much more important than susceptibility towards heat It is of course possible that evolutionary selection of the structure was in response to pressures other than stability and coincidentally resulted in DNA with high stability to metal ion degradation

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Amounts of isoaccepting lysine tRNAs change with the proliferative state of cells

VARIATIONS in isoaccepting tRNA populations occur in mammalian cells during development, neoplasia, and virus infection, and such changes may reflect the involvement of tRNA in regulatory processes[1,2], although firm evidence to that effect is not available Ortwerth and Liu[3] have shown that in normal or neoplastic cells an isoaccepting species of lysine tRNA, $tRNA_4{}^{Lys}$, is present in dividing cells but absent from non-dividing cells Ortwerth *et al*[4] determined some of the functional properties of $tRNA_4{}^{Lys}$ from various tissues and obtained evidence that the species is a real isoacceptor of $tRNA^{Lys}$ rather than an artefact Normal, proliferating cells that were examined had only relatively small amounts of $tRNA_4{}^{Lys}$, however, a larger proportion of the isoacceptor was found in neoplastic cells such as mouse leukaemia and Morris hepatoma cells It is desirable, therefore, to establish whether the large amount of $tRNA_4{}^{Lys}$ is peculiar to the neoplastic state or whether normal cells may also have large amounts of that isoacceptor under some growth conditions Therefore, we studied isoaccepting lysyl-tRNA profiles from mouse cells in different states of proliferation adult liver, embryo and growing and quiescent primary cultures of embryonic cells We found that normal growing cells also have an appreciable amount of $tRNA_4{}^{Lys}$

Ten-day-old mouse embryos, with heads and extremities removed, were prepared for monolayer tissue culture by dispersing cells with trypsin and seeding in roller bottles in Eagle's MEM[5] containing penicillin G (100 U ml⁻¹), streptomycin (100 µg ml⁻¹) and 10% foetal calf serum tRNA and aminoacyl-tRNA ligases were isolated from primary cells, embryo and liver as previously described[6] and tRNA was purified further by DEAE–cellulose chromatography[7] tRNA was aminoacylated with lysine as before[8]

In mouse leukaemia cells in suspension culture, $tRNA_4{}^{Lys}$ is 47% of the total $tRNA^{Lys}$, and this amount decreases to 16% as cell density increases and proliferation stops[3] Comparable data on the amount of $tRNA_4{}^{Lys}$ in a uniform population of rapidly dividing normal cells and the effect of increasing cell density were not available We have compared the Lys-tRNA^{Lys} profiles of growing and density-inhibited quiescent primary cultures of embryonic cells The results (Fig 1a) indicate a much larger amount of $tRNA_4{}^{Lys}$ in growing cells than in resting cells Consistent with these results is the finding of an appreciable amount of $tRNA_4{}^{Lys}$ in embryonic cells which were not in tissue culture (Fig 1b) These results also indicate that placing the cells in tissue culture does not in itself lead to an altered tRNA^{Lys} profile In contrast to results with proliferating cells, $tRNA_4{}^{Lys}$ is present only in a very small amount in adult mouse liver (Fig 1c)

The results are summarised in a quantitative manner in Table 1 $tRNA_4{}^{Lys}$ varies from 3% in adult mouse liver to 24% in proliferating monolayer culture Similar results with cultured murine sarcoma virus-transformed cells (H J , D J , and C H , unpublished) and SV40-transformed cells (J Katze, personal communication) have been obtained In both cases, $tRNA_{5a}{}^{Lys}$ (ref 8) seems to be present in addition to $tRNA_4{}^{Lys}$ in rapidly proliferating cells and decreases as the cell population becomes

Table 1 Effect of state of growth of cells on chromatographic distribution of isoacceptors of lysine tRNA

Cells	Growth state	Distribution of lysine tRNA isoacceptors			
		1	2	4	5
Adult mouse liver	Quiescent	−	58	3	38
Primary mouse embryo	Quiescent	2	50	9	40
Mouse embryo	Growing	4	37	15	44
Primary mouse embryo	Growing	2	35	24	38

Fig. 1 Comparison of lysyl-tRNA from primary embryo cells in growing and quiescent states, from adult liver and from whole embryo Growing primary cells were collected at $5\ 5 \times 10^4$ cells cm^{-2}, and quiescent primary cells were collected 48 h later at $9\ 4 \times 10^4$ cells cm^{-2} The generation time for primary cultures of mouse embryo cells is about 18 h Lysyl-tRNA was chromatographed in the RPC-5 system[9] on a $0\ 9 \times 100$ cm column with a linear gradient (500 ml) from 0 5 to 0 65 M NaCl in 10 mM sodium acetate buffer, pH 5, containing 10 mM MgCl$_2$ Fractions of 4 ml were collected at a flow rate of 1 3 ml min^{-1} Radioactivity in each fraction was determined in 15 ml of a 2 1 mixture of toluene-base scintillation fluid and Triton X-100 tRNA$_3^{Lys}$ is a minor species and is not resolved in our system a, Growing primary cells (- - - -, ^{3}H) and quiescent primary cells (———, ^{14}C), b, growing primary cells (- - - -, ^{3}H) and embryo (———, ^{14}C), c, growing primary cells (- - - -, ^{3}H) and liver (———, ^{14}C)

dense (The presence of tRNA$_{5a}^{Lys}$ is not always detected by RPC-5 chromatography, but benzoylated DEAE–cellulose chromatography provides resolution[8]) The effect of cell density on profiles of other isoaccepting tRNAs has been noted[10]

It is now clear that the distribution of isoaccepting species of tRNA^{Lys} in both normal and neoplastic cells varies with the proliferative state of cells The constancy of the sum of percentages of tRNA$_2^{Lys}$ and tRNA$_4^{Lys}$ suggests that species 2 and 4 are structurally related with differences attributed to the degree of modification[4] Confirmation of that possibility and investigations into functional differences are the subjects of continuing investigations

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Errata

In the article "An ancient lunar magnetic dipole field" by S K Runcorn (*Nature*, **253**, 701, 1975) the legends to Fig 2a and b were transposed

In the article "A gene cluster in *Aspergillus nidulans* with an internally located *cis*-acting regulatory region" by H N Arst and D W MacDonald (*Nature*, **254**, 26, 1975) there was an error in Fig 1 The two arrows linking L-proline and L-Δ^1-pyrroline-5-carboxylate should be reversed—so that the top arrow points to the right and the bottom to the left

matters arising

Cooling velocity and cell recovery

FARRANT et al[1] have suggested that two separate processes determine the survival of cultured cells thawed from −196 °C storage, namely a holding temperature of about −26 °C preceding the subsequent storage temperature We report that optimal recovery of cultured cells after freezing depends on an optimal cooling velocity[2,3] rather than temporary attainment of a particular subzero protective temperature zone[1] before storage in liquid nitrogen at −196 °C

Normal human diploid fibroblasts were cultured in Dulbecco's modified Eagle's (DME) medium with 16 6% foetal calf serum, 20 mM HEPES buffer, penicillin, streptomycin and neomycin Cells were suspended and dispensed into glass ampoules (cryules, Wheaton Glass Company) each containing 10⁶ cells ml⁻¹ of medium and either 5% or 10% DMSO as described previously[3]

In pilot experiments, using an electric thermometer whose tip reached into an ampoule filled with 1 ml of the cryoprotective medium, we measured the time required to cool an ampoule from +25 °C to −25 °C This was done either by immersion of the ampoule in liquid nitrogen followed by rapid transfer at −25 °C to a methanol bath set at −26 °C or immersion in the methanol bath directly The time required was 30 s or 2 5 min, respectively

We designed the freezing experiments as follows (1) rapid cooling from +25 °C to −25 °C within 30 s followed by maintenance at −26 °C for 1 h, (2) slow cooling from +25 °C to −25 °C within 2 5 min followed by maintenance at −26 °C for 1 h, (3) slow cooling from +25 °C to −25 °C within 2 5 min followed by immediate immersion in liquid nitrogen After 1 h, the ampoules of series (1) and (2) were also rapidly immersed in liquid nitrogen All experiments were performed with two different concentrations of DMSO (5% and 10%)

Table 1 shows survival of only 0 75% of the cells cooled rapidly to −26 °C (cooling velocity about 100 °C min⁻¹) and maintained there for 1 h before immersion in liquid nitrogen, 52 7% survival when cooled more slowly to −26 °C (cooling velocity of about 20 °C min⁻¹) and then immediately immersed in liquid nitrogen, 61 2% survival after cooling slowly to −26 °C and maintained at this temperature for 1 h before transfer to liquid nitrogen

We have previously shown that optimal recovery of frozen human diploid fibroblasts from storage at −196 °C is achieved if the cooling velocity at subzero temperatures down to −30 °C is about 1 5 to 4 5 °C min⁻¹ (ref 3) Furthermore, cells do not cool at a constant rate in the range of +10 °C and −30 °C (Fig 1)

The assumption that the freezing velocity is the most important parameter for recovery agrees with Farrant et al[1], except for one experimental detail They state that (starting at room temperature) −26 °C had been reached in less than 1 min, while optimal recovery of frozen

cells required at least 5 min immersion in a bath at −26 °C This is inconsistent with our results (experiment 1) and does not demonstrate conclusively the existence of a protective temperature zone As our results show, on the one hand, nearly optimal recovery can be reached without keeping cells at a protective temperature zone (experiment 3) and on the other almost no viable cells remain even though they were kept at a recommended protective temperature for 1 h if the initial freezing rate was too high

Farrant et al[1] happened to apply the optimal cooling rate for their cells when they immersed their glass test tubes for some time at −25 6 °C, whereas −22 °C,
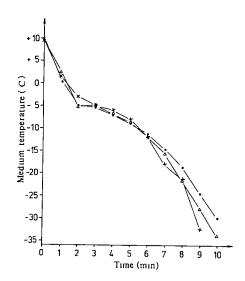
Fig 1 Cooling rates of three independent experiments using ampoules containing 1 ml of tissue culture medium

−30 °C, and −40 °C provided less optimal cooling rates Larger sample volumes cooled in polypropylene tubes rather than glass recovered less well at −25 6 °C, because Farrant et al[1] were working outside the optimal cooling velocity for their particular cells We have described elsewhere that the cooling velocity can be controlled well by different types of insulation of the vial used for freezing[3]

As shown previously[2,3], the optimal cooling velocity differs between lymphocytes, red blood cells, Chinese hamster

Table 1 Survival rate of human diploid fibroblasts following different freezing procedures

DMSO in medium (%)	Time for cooling +25 °C to −26 °C	Time held at −26 °C	Number of cells recovered		Recovery rate (%)
			Viable	Non-viable	
5	30 s	1 h	5,084± 1,987	752,230± 78,352	0 67
10	30 s	1 h	5,698± 2,358	676,523±106,958	0 83
5	2 5 min	1 h	448,818±26,010	296,360± 38,823	60 22
10	2 5 min	1 h	524,235±73,263	318,570± 52,770	62 20
5	2 5 min	0	369,737±82,016	340,845± 42,823	52 03
10	2 5 min	0	463,636±55,812	398,483± 9,061	53 34

All vials were immersed rapidly in liquid nitrogen after they had reached −26 °C according to the schedule given above Cells were thawed rapidly to +37 °C after about 1 h, medium containing DMSO was removed by centrifugation and each ampoule seeded into one 25 cm² culture flask (Falcon Plastics) containing 5 ml DME culture medium Cell survival was measured as percentage attached (viable) cells compared with floating (non-viable) cells after incubation for 15 h at +37 °C (ref 2) Each figure represents the mean of four independent determinations

cells, human diploid fibroblasts and others but the principle is the same

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DR FARRANT *et al* REPLY—We believe that the protection of living cells against freezing damage given by cooling rate techniques or by a period at a constant subzero temperature are both due to the effects on the cells of the time of exposure to the subzero conditions

We have evidence that a continuous reduction in temperature (cooling rate) is not essential for protection The conclusive point is the onset of protection against subsequent rapid cooling damage with time at a subzero holding temperature once that temperature has been reached This can be seen (1) in our data[1], where the recovery of lymphocytes and tissue culture cells thawed from −196 °C increased with time (between 1 and 10 min) after −26 °C had been reached, (2) in other work on increases in protection with time at a constant subzero temperature in spermatozoa[2,3], red blood cells[4], platelets[5], tissue culture cells[6], nematodes[7], and mulberry twigs[8], (3) from unpublished work with Chinese hamster cells cooled in capillary tubing to −26 °C within 17 s, in which protection against rapid cooling to −196 °C is absent for the first 1 min and yet increases to a maximum at 2 5 min, and also (4) from the data of Rudiger *et al*[9], where there is an improvement in survival of diploid cells with time at −26 °C

The argument put forward by Rudiger *et al*[9], that cooling rate is essential for protection depends solely on their data showing very low survival of the cells cooled to −26 °C within 30 s It is possible that cooling a 1-ml sample with liquid nitrogen and relying on an 'electric thermometer' of unspecified thermal mass to determine when the sample has reached −26 °C (ref 9), in fact cools regions of the cell suspension to a much lower temperature, thus producing intracellular ice before the period at the holding temperature This should be checked in

control samples thawed immediately from the holding temperature, which in our experiments gave high survival Also, with a different cell type, amount of cryoprotective additive (including serum) and the slower thawing rate unavoidable with a larger sample, the optimal conditions of time and holding temperature are different (our unpublished work) The conditions we described originally[1] therefore do not constitute a universal recipe

In short, cooling rate is important but survival using cooling rate techniques can be explained in terms of the cells being exposed for different times to different subzero temperatures however, protection acquired with time at a constant subzero temperature cannot be dependent on cooling rate

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Long-term periodicities in the sunspot cycle

COHEN and LINTZ[1] claim that their work shows the presence of a 179-yr periodicity in sunspot cycles, and that this is a beat phenomenon Another method of analysis can be used to demonstrate more clearly that a period of approximately this length exists in the data, and is indeed the result of beating between two more rapid oscillations Cohen and Lintz used the term "beat frequency" in the sense of half

the difference frequency, but here the beat frequency will be taken to be the difference frequency Whatever definition is adopted, successive maxima in the beats resulting from frequencies with periods 10 9 and 9 7 yr (ref 1, Fig 2) occur at 83-yr intervals, taking the frequency separation to be 0 012 cycle yr⁻¹ as they do The 167-yr interval mentioned by Cohen and Lintz is approximately a waveform repetition period

To study beat phenomena it is advantageous to consider the transformed sunspot numbers obtained by changing the sign of the numbers for alternate cycles in the way proposed by Bracewell[2] and others Cole[3] has carried out a spectral analysis on such data by standard methods and the results in his Fig 1 shows that the resulting power spectrum is much simpler than that obtained by using the ordinary, positive, sunspot numbers It should therefore be much easier to make deductions about the behaviour of sunspot numbers from this type of spectrum For instance, as Cole's spectrum (in his Fig 1*d*) shows three main peaks, the largest with a period of 22 2 yr and two smaller ones with periods of 19 7 and 18 3 yr, one would expect to find beat frequencies with periods of 175 and 104 yr in the data

If one uses the ordinary sunspot numbers, any period appearing in the envelope of the maxima will also appear as that of a genuine spectral component because the envelope of the sunspot minima is almost constant (nearly zero) This need not happen if the transformed sunspot numbers are used, and indeed does not happen in Cole's spectrum Therefore the 175-yr period in the maxima is certainly a beat phenomenon

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Matters arising

DRS COHEN AND LINTZ REPLY—We had hoped that use of the term "beat" would help the casual reader to better understand the phenomena observed Given the confusion that has arisen, however, we probably should have discussed the sunspot cycle only in terms of the waveform repetition period (or frequency) In doing so, the data indicate that the waveform should repeat about every 179 yr

Specifically, through correlation analysis, and guided by the periods of significant signatures in the maximum entropy (MESA) spectrum for the data interval 1750–1963, we find that the spectrum of the 12-month smoothe

sunspot numbers shows components at 89 6, 57 1, 11 2, 9 9 and 8 1 yr We estimate, therefore, that to a first approximation, the repetitition period for a waveform produced by the superposition of sinusoids having these periods is about 179 yr $(2 \times 89\ 6 = 179\ 2, \quad 3 \times 57\ 1 = 171\ 3, \quad 16 \times 11\ 2 = 179\ 2, \quad 18 \times 9\ 9 = 178\ 2, 22 \times 8\ 1 = 178\ 2)$

That an 89 6-yr component is found in the spectrum suggests that the Sun may be behaving as a nonlinear (square law) device, passing not only the two major driving frequencies (11 2 and 9 9-yr periods), but also generating components corresponding to twice the driving frequencies, and to the sum and difference frequencies Thus, we might expect to find spectral components at the following frequencies

$$
\begin{aligned}
f_2 - f_1 &\quad 89\ 6\ \text{yr} \\
f_1 + f_2 &\quad 5\ 3\ \text{yr} \\
2f_1 &\quad 5\ 6\ \text{yr} \\
2f_2 &\quad 5\ 0\ \text{yr}
\end{aligned}
$$

Weak, but significant, components are found in the 214-yr MESA spectrum at periods of 4 8, 5 5 and 5 8 yr (correlation analysis yields period estimates of 4 8, 5 3 and 5 8 yr) and these may represent the expected heterodyne components

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1 Cohen, T J, and Lintz, P R, *Nature*, 250, 398–400 (1974)

Population stratification as an explanation of IQ and ABO association

A RECENT study[1] of English villages claims to have shown significant differences in mean IQ between some of the ABO blood group phenotypes The population can be divided into two groups, namely those who were born locally and those who were born elsewhere, henceforth referred to as locals and non-locals respectively There is a significant difference in ABO frequencies between the two groups The frequency of ABO phenotypes varies significantly[2] throughout England, so that differences in frequencies between the local and non-local populations are not surprising Also, the non-locals have significantly higher mean IQs than the locals, regardless of the blood group phenotype The authors[1] claim that "There is thus genetic evidence for at least two population groups in the area, but this alone cannot be responsible for the association between ABO blood group and IQ which the analysis reveals "

We should like to suggest that the observed association could in fact be the result of this stratification of the population The combination of the difference in ABO frequencies and the difference in

mean IQ between the local and non-local groups causes an association This is explained below in a very simple model

The population is divided into two groups, local and non-local Each group is subdivided into, for example, blood groups, which will be labelled 1, 2, and 3 The population frequencies of blood group i are p_i and q_i for locals and non-locals respectively

$$
(i = 1,2,3, \sum_{i=1}^{3} p_i = 1 = \sum_{i=1}^{3} q_i)
$$

The total sample size of locals is N_1 and non-locals N_2 (Table 1) Define $M = N_1 / (N_1 + N_2)$

Denote the mean IQ of locals by x_1 and that of the non-locals by x_2 (independent of blood group) Then the mean IQ of blood group 1, denoted a_1 is given by

$$
a_1 = \frac{N_1 p_1 x_1 + N_2 q_1 x_2}{N_1 p_1 + N_2 q_1}
$$

$$
= \frac{M p_1 x_1 + (1-M) q_1 x_2}{M p_1 + (1-M) q_1}
$$

Similarly the mean IQ of blood group 2 is

$$
a_2 = \frac{M p_2 x_1 + (1-M) q_2 x_2}{M p_2 + (1-M) q_2}
$$

So

$$
a_1 - a_2 = \frac{M(1-M)\,(x_1 - x_2)\,(p_1 q_2 - q_1 p_2)}{(M p_1 + (1-M) q_1)(M p_2 + (1-M) q_2)}
$$

Thus $a_1 \neq a_2$ if $x_1 \neq x_2$ and $p_1 q_2 \neq q_1 p_2$ So if the mean IQ of the locals and non-locals is different and their blood group frequencies are also different, then in the total population the mean IQ of each blood group will be different

Is this sufficient to explain the difference observed in the Otmoor study? The appropriate parameter values are obtained from Tables 4 and 5 of that paper[1] They are $p_1 = 0\ 5238$, $q_1 = 0\ 3869$, $p_2 = 0\ 3691$, $q_2 = 0\ 4696$, $p_3 = 0\ 1071$, $q_3 = 0\ 1435$, $x_1 = 100\ 671$, $x_2 = 110\ 965$ (Blood group 1 refers to the A_1 phenotype and 2 to O) The values of N_1 and N_2 in those Tables 4 and 5 are different But the same general result holds for both sets of numbers The values in Table 4 will be used as they are closest

Table 1 Numbers observed in each subgroup

Blood group	Local	Non-local
1	$N_1 p_1$	$N_2 q_1$
2	$N_1 p_2$	$N_2 q_2$
3	$N_1 p_3$	$N_2 q_3$

to the total used in Table 1 Thus $N_1 = 168$, $N_2 = 460$ so $M = 0\ 2675$

Using this model we estimate that the mean IQ of A_1 phenotypes in the total

population sampled would be 107 56 and the mean IQ of O phenotypes 108 67 Thus the expected difference in mean IQ between these two blood groups, for this particular population, is 1 11 (The standard error of this estimate is 0 15 so that the difference predicted is significantly different from zero) The observed values of 106 95 and 109 75 for A_1 and O respectively are not significantly different from these expected values Although the observed difference of 2 8 IQ points is significantly different from zero it is not significantly different from the estimated expected value of 1 11 Thus the stratification of the population would be an adequate explanation of the observed differences in IQ between the blood groups

The model described above does not take account of the observed sex differences in IQ and ABO frequencies, but can be simply extended to do so In this case the expected difference in IQ between the A_1 and O phenotypes is 1 2 and the same general result applies

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GIBSON, HARRISON and HIORNS REPLY—We appreciate the model proposed by Thomson and Bodmer and accept that its application to the Otmoor data makes the hypothesis that the association we reported between ABO blood group status and IQ is caused by the presence of two population groups in the area (locally born and non-locally born), more likely than we originally thought We still however consider that this explanation as we originally stated, is unlikely to be the sole cause for the association on the following grounds

On the basis of the model, the ability of a test to detect IQ differences between people of blood groups A_1 and O ought to be greatest in those villages where the proportions of locals and non-locals are most equal There is no relationship between this proportion and the level of association in the various villages Indeed, in Beckley and Horton, where the sample contained the second highest proportion of locally-born subjects, the

phenomenon was not detectable and the actual differences between A_1 and O were in fact the other way round from those found in the other villages

Although regional differences in the frequency of ABO genes certainly occur, there is no evidence in the data of Kopec[1] for micro-differentiation between neighbouring Oxfordshire groups from which most of the non-local individuals came Further the migration matrix analysis of movement into the Otmoor populations[2] not only predicted that there would be no between-village heterogeneity but that the genetic composition of the villages should be the same as those in the surrounding neighbourhood It therefore seems unlikely that the differences in ABO frequencies between locals and non-locals arise from their being two primary populations (We should perhaps add here that further analysis of other polymorphic systems indicates genetic heterogeneity between the Otmoor villages, but this is not shown by the ABO system)

An overall analysis of the IQ variance according to the ABO status reveals significant heterogeneity In the four groups, local males, local females, non-local males and non-local females the IQ variance is always smaller for O subjects than for A_1 ones Although these differences do not reach individual significance, an analysis of variance of logarithms of variances according to blood group status, sex and origin, indicate significant differences by AO status and sex ($t = 2\,23$ and $2\,03$ respectively with 629 d f) (This variance analysis includes additional information to that previously reported, but the same trends are present in the original data) The two-population model cannot account for variance differences but selective migration can If, for example, high IQ individuals tend to out-migrate from Otmoor, which seems likely, and if these outmigrants tend to have a relatively high frequency of blood group O, then one would find a comparatively low variance of IQ in those O individuals who remained and were tested Selective migration of this form could also explain the differences in the ABO frequency of the locally and non-locally born groups and particularly, the unusually low frequency of the O gene in the locally born males

It is perhaps also worth mentioning that in some of the village groups a significant difference in the IQ of A and O people also exists among the non-locals

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Dantrolene sodium and 'skinned' muscle fibres

HAINAUT and Desmedt[1] have reported the results of their work with Dantrolene sodium (1- {[5-(P-nitrophenyl) furfurylidine] amino} hydantoin sodium hydrate salt) and skeletal muscle In single fibres of frog semitendinosus muscle they showed a greatly reduced twitch force and a shift in the dose–response curve for potassium contractures These results in general agree with those already obtained with whole muscle[2] Other workers[3] have shown that Dantrolene sodium has no effect on sarcolemma resistance or capacitance, and all evidence[2-4] points to the intracellular Ca^{2+} control mechanisms as the level at which Dantrolene sodium produces its effect

If the contractile proteins themselves are not involved, the twitch tension following a single release of Ca^{2+} from the sarcoplasmic reticulum may be reduced in two ways less Ca^{2+} may be released, and also the rate of uptake of Ca^{2+} may be raised Hainaut and Desmedt[1] show a reduction of 30% in the aequorin luminescence of barnacle fibres, following treatment with high concentrations of Dantrolene sodium, thus demonstrating that Ca^{2+} release is certainly reduced in this muscle In the frog, however, the twitch may be reduced by 75% with 15 μM Dantrolene sodium and it is possible that several mechanisms may be involved A good method for detecting changes in Ca^{2+} uptake into the sarcoplasmic reticulum is the Ca^{2+}–gel pipette technique of Gillis[5], which can be used for frog muscle Here, controlled local contractions are produced by a short contact with the Ca^{2+}-containing gel on an area of the muscle fibre from which the sarcolemma has been mechanically removed Thus Ca^{2+} is introduced from an external source, and relaxation follows with the uptake of these ions into the sarcoplasmic reticulum Drops of Dantrolene sodium in aqueous solution were added to the fibre under paraffin oil The concentration of Dantrolene sodium was about 15 μM, a saturated solution in 120 mM KCl In this 'skinned' preparation with no membrane it may be expected that the concentration of the negatively charged Dantrolene ions within the sarcoplasm is much higher than that inside an intact fibre The volume of Dantrolene sodium solution given was roughly twice the volume of the 'skinned' region of the fibre, so it would be expected that the concentration at the point of the test contractions remained high for some time

Contraction–relaxation cycles before and after Dantrolene sodium treatment were recorded on cine film and compared for speed and distance of contraction, spread of activation and rate of relaxation

There was absolutely no change in any of these parameters, either a few seconds after Dantrolene sodium or several minutes later In view of the undoubted effects of Dantrolene sodium on the intact muscle it would seem that it acts uniquely on Ca^{2+} release—the event bypassed using the Ca^{2+}–gel pipette It is clear that sliding filament reactions and Ca^{2+} uptake are not altered in the 'skinned' fibre Also, as the spread of activation was not reduced it seems likely that the regenerative release of Ca^{2+} from the reticulum is not inhibited This aspect of the possible actions of Dantrolene sodium has been discussed previously[4] in relation to other results

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DRS HAINAUT AND DESMEDT REPLY— Studies on single muscle fibres have demonstrated that Dantrolene sodium reduces the Ca^{2+} release from the sarcoplasmic reticulum during a twitch[1] The finding that Dantrolene sodium does not accelerate the myoplasmic Ca^{2+} uptake studied in skinned muscle fibres[2] is in line with our observations on intact barnacle muscle fibres injected with aequorin Figure 2d–e from our paper[1] indeed showed that the calcium transient, though markedly reduced in size, was not significantly changed in its time course by Dantrolene sodium The half time of the exponential falling phase of the luminescence transient studied for different depolarisations in six different fibres was 38 9 ms (s d = 1 8 ms) before and 38 4 ms (s d = 2 9 ms) in presence of Dantrolene sodium These and other data (K H, and J E D, unpublished) confirm that Dantrolene sodium depresses the Ca^{2+} release but does not affect the rate of reuptake of Ca^{2+} in intact muscle fibres

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reviews

Sides of Bacon

A MEASURE of Francis Bacon's influence and importance to the history of ideas lies in the number of ways in which his works can be studied, and the number of books written about him. Not so long ago it was the fashion to emphasise his modernity and see in his writings the nascent scientific revolution of the 17th century. Now there is a useful corrective in a tendency to regard him as a Renaissance man (he was three years older than Shakespeare and Galileo) and to examine his thought in terms of 16th century concepts.

Out of this latter tendency comes Dr Jardine's extremely learned and lively study, based upon a scholarly examination of the dialectical tradition to which Bacon was exposed at Cambridge and which, as Dr Jardine shows, he never entirely shook off. There were in the later 16th century many new treatises on the art of discourse, intended to teach the young student both the art of logical argument and its application in rhetoric. Here we see how Bacon duly followed their precepts in all his works to some extent, most especially in his literary writings and in his logic; it is useful to be reminded what is easy to overlook, that the *Novum Organum* is first and foremost a logical or dialectical work, and only secondarily scientific.

This said, it must be added that this is not the whole of Bacon; this Bacon is studious, scholarly, derivative, pedantic, and eminently forgettable. There is another Bacon, who transcended his

Francis Bacon : Discovery and the Art of Discourse. By Lisa Jardine. Pp. viii+267. (Cambridge University Press: London, 1974.) £4.90; $15.50.

sources and his dialectical methods of thought to fire the imagination of budding natural philosophers born after (sometimes long after) he wrote. It is revealing to consider Bacon's elaboration of forms in terms of his dialectical education, but it provides neither the whole meaning nor any of the relevance of his work to the advancement of science. Dr Jardine is eminently successful in showing the traditional content; her approach cannot and does not help us to understand why it was important to later natural philosophers that Bacon said of heat that it was "a motion, expansive, restrained, and acting in its strife on the smaller parts of bodies". Nor does it allow us to appreciate that it was relevant to the development of the mechanical philo-

sophy that Bacon connected the "form of whiteness" with the reflectivity of the small particles of bodies. The scientist and historian of science looks favourably upon Bacon's remark : "A leaden ball of a pound weight dropped from a tower reaches the ground in (say) ten seconds : will a ball of two pounds in weight (in which the force of natural motion, as they call it, ought to be doubled) reach the ground in five seconds? No, but it will take almost the same time in falling, and will not be accelerated in proportion to the increase of quantity". Surely this is close to the heart of advancing science in 1623.

It may be true, as Dr Jardine says, that "in treating systematic experimenting as subsidiary to a formal logic which manipulates essential definitions . . . Bacon dismissed as secondary and provisional much of what we now regard as science"; but did he dismiss so much of what was then called natural philosophy? Rather, may it not be the case that his casting of new ideas in an old framework and his use of the language and methods of thought in which his readers in the next two generations were as thoroughly trained as he had been, made these ideas easier to understand, and certainly no less effective?

But modern readers, not so educated, are less able to appreciate the 16th century context of Bacon's thought and, above all, expression. Dr Jardine's book is valuable in providing understanding. **Marie Boas Hall**

THIS volume is the first of a projected series to be based on courses of lectures given at the Harwell Education Centre, by lecturers both from Harwell itself, and from outside. These Harwell lectures have been generally at an introductory level, and the present volume is in line with that trend. This description is in no way to be construed as an implicit criticism, for there has been a clear need for just such a volume. According to the preface, "the principal aim of the book is to provide a balanced and integrated account of instrumental methods (used for the characterisation of materials) for those who carry responsibility for research programmes and who must be able to judge the potential contribution from alternative techniques, and for specialists in any one technique who require an overview in adjacent fields of work".

The book achieves these aims effectively, especially the first; the second category of readers may find some (not all) of the chapters somewhat elementary for their requirements. The book

The stuff tis made of

Modern Physical Techniques in Materials Technology. (UKAEA Research Group—Harwell Series.) Edited by Mulvey, T., and Webster, R. K. Pp. xiv+321. (Oxford University Press: London, November 1974.) £9.50.

is also well pitched for an introductory course in a materials science course at university; I am already using it for that purpose.

Of the 20 chapters (8 by Harwell staff), 4 deal with various forms of

diffraction, 7 with micrographic methods (with a heavy emphasis on electron instruments), 7 on diverse methods of chemical analysis by physical means (optical emission and absorption spectrometry; electron-probe microanalysis; Auger, X-ray and mass spectrometry; activation analysis) and 2 with Mössbauer and magnetic resonance spectroscopy, regarded primarily as means of chemical identification.

Loosely, the book offers advice on how to find out what it looks like and what it's made of. The advances of the last 15 years have changed these twin arts out of all recognition, and this book offers a useful outline of what is now feasible. Some of the techniques are still difficult enough to oblige the wise casual user to contract the work out: Harwell is ready and waiting. **R. W. Cahn**

Span Quaternary fields

Pedology, Weathering, and Geomorphological Research. By Peter W. Birkeland. Pp. xiii+285. (Oxford University Press: London and New York, October 1974.) £6.25.

FEW books set out to bridge the considerable gaps between the various disciplines in Quaternary studies. This may be a reflection of the range and diversity of material, and consequently of the specialisms demanded of the author. It must, therefore, have required a commendable boldness to embark on a work as complicated as that suggested by the title of Professor Birkeland's new book.

The text spans the interesting and fruitful field between pedology and other Quaternary studies, but each reader will probably have a different view of the likely contents, and may therefore be frustrated by what is actually covered. The title does not represent the emphasis accurately. The major stress lies on the factors of soil formation (chapters 6–11), but there are introductory chapters on soil profiles, properties and classification (chapters 1 and 2), a section on weathering and soil forming processes (chapters 3–5), and a final single chapter on the use of soils in Quaternary studies. Pedologists will be familiar with most of these topics, and anyone interested in weathering and soil processes will find the material rather brief but succinct.

Geomorphologists may be thwarted in their search for appropriate material as there is no chapter specifically upon geomorphological work. Topics of recent geomorphic interest, such as quantitative research into processes, slope analysis and chronology are barely mentioned. The level is more appropriate to senior undergraduate and first year postgraduate students in British universities.

The text is geared to the American market. Nearly all the examples are taken deliberately from North America, and it is a matter of some regret that there are relatively few publications discussed, even in English, from outside that area. The depth of recommended reading varies from 73 references for one chapter down to 11 for another.

The lack of a review of biological factors leads to some imbalance; neither the chapter on vegetation and soil relationships (which fails to consider either fauna or microorganisms), nor the final chapter give adequate consideration to the biotic influences on soil development.

Despite these general criticisms and a number of minor quibbles, this book makes worthwhile reading on several counts. It does present a summary of recent (particularly American) research and it does bring together the research of diverse but related fields. Amplification of the topics presented, and a more extensive review of allied geomorphological research would add to the value of any future editions.

Peter A. Furley

Unwitting participant: helping scientists to study the ability of penguins to survive in icy wastelands. From *The World Science Year Book, 1975.* Pp. 441. (World Book Encyclopedia Incorporated: Chicago, 1975.) n.p.

Bubble devices

Magnetic Bubbles. By T. H. O'Dell. Pp. x+159. (Macmillan: London and Basingstoke, January 1975.) £8.50.

MAGNETIC BUBBLES will prove invaluable in a variety of roles; it will, for example, provide an excellent introduction and foundation course for device engineers and materials scientists entering the new field of magnetic bubble devices. In spite of the rapidly growing bibliography on magnetic bubbles the extensive references provided by the author will considerably aid established workers in this field to recover previously published information. The author has concentrated on the underlying physics of both the operation of devices and the materials used. The treatment has emphasised, where appropriate, the mathematical aspects of the subject, and in most cases it has been done in such a way as to inspire confidence, maintaining a balance between the basic philosophy of bubble devices and a rigorous mathematical treatment.

Consider a thin layer of magnetic material which, because of its structure, can easily be magnetised to saturation only in directions normal to itself. A magnetic bubble is a small and mobile cylindrical region, extending through the layer, which is magnetised in a sense opposing the remainder of the layer. Bubbles are used for data processing and storage, each one representing a '1' digit. A bubble device consists simply of an assembly of a number of integrated circuits each of which carries magnetically activated tracks, that is, shift registers, along which are driven patterns of bubbles and gaps representing binary data.

Chapters on magnetostatics and bubble dynamics are particularly well presented, bringing together a well balanced explanation incorporating a number of basic concepts which previously have been scattered among a handful of technical articles. These chapters would be valuable in aiding a first degree course in magnetics.

Two chapters deal with areas close to device design and technology. One provides a review of the range of magnetic materials which have been studied as candidates for bubble devices and explains the important characteristics which ensure adequate bubble stability. Although sufficient detail is given on garnet layers—which are prepared by liquid phase epitaxy—to indicate that they represent a viable device basis the author has omitted details on the epitaxy process itself. That is a pity because were such detail included it would be clear that the models used to represent the layers are idealised in that available layers actually possess nonuniformities in composition throughout their thickness. The other sections dealing with devices provide an excellent treatment of bubble motion in the potential wells produced by the shift register elements. The author's approach is, I believe, unique and is certainly likely to inspire the device engineer.

This review would be incomplete were I not to report the one error I found. Although nothing which follows it is invalidated, the approximation used by the author in Chapter 3 for the exchange constant may mislead readers to assume that the exchange constant is virtually independent of temperature. Actually, it decreases monotonically with increasing temperature, reaching zero at the Curie temperature.

Anthony Marsh

Vertebrate History: Problems in Evolution. (McGraw-Hill Series in Population Biology.) By Barbara J. Stahl. Pp. ix + 594. (McGraw-Hill: New York and Maidenhead, 1974.) £8.75.

THIS is the most interesting book on this subject that I have read, because it deals with the problems involved in understanding the course of vertebrate evolution. Most textbooks on vertebrate history are primarily accumulations of information on the structure and diversity of each group of fossil vertebrates, and are really textbooks of palaeo-osteology. Yet, for any reader, the interest of a subject lies in the problems it contains, in the lines of evidence available for solving those problems, and in the methods that can be used for obtaining and analysing that evidence. For example, the problem of the origin of the amphibians is not merely one of osteological modification; the papers that have discussed this subject also include opinions based on studies of the embryology, life history and behaviour of present-day amphibians, the anatomy and physiology of respiration, the ecology, climatology and geography of the Devonian, and the nature and distribution of Devonian sediments. Consideration of such wider topics in turn forces the reader to ponder to what extent they may or may not be relevant to the central question. It provides, therefore, a far better methodological training than the normal textbook description of the trees to the exclusion of the wood they make up.
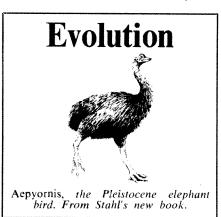
The book is divided into nine main chapters: fossils (their nature, discovery and investigation); the origin of the vertebrates; bone and cartilage in early vertebrates; the first fishes with jaws; the rise of the modern fishes; the amphibians and their origin; the rise and fall of the reptiles; birds; and finally, mammals. The mammals are thus cut down to their proper status—in most books on vertebrate palaeontology, which give a description of all the diverse orders of mammal, they occupy one third to one half of the text.

The chapters themselves are written in a style that is concise and enjoyable to read, and the concepts and questions that arise are clearly defined. The format is attractive, with topic headings to the side of the text. In considering the main problems raised in these chapters, Dr Stahl has had to read and evaluate a large number of papers. The most important of these are listed in a well-selected bibliography of about 280 titles (some as late as 1972), and there are 216 excellent figures.

Dr Stahl's book cannot replace such textbooks as those of Romer or Colbert as a source of information on vertebrate palaeontology. It does, however, provide an opportunity for the interested student to find out more without becoming surfeited by facts, and thus to come to appreciate the proper significance of the information those other textbooks provide.

Barry Cox

Evolution

Aepyornis, *the Pleistocene elephant bird. From Stahl's new book.*

The Genetic Basis of Evolutionary Change. By R. C. Lewontin. Pp. xiii + 348. (Columbia University Press: London and New York, 1974.) £2.15.

FOR Darwin, evolution was the conversion of variation among individuals within an interbreeding population into variation between species and genera. The central ideas of molecular biology did not have an immediate impact on evolutionary thought. They did change our concept of mutation from a process of shuttling back and forth between wild-type and mutant states to that of an infinite series of changes which almost never back-tracks. But the full impact waited on the development of two experimental techniques: the determination of amino acid sequences in peptides, and gel electrophoresis. These techniques provided for the first time quantitative answers in genetic terms to two important questions—how fast has evolution proceeded, and how much genetic variation is there in natural populations? The classical evolutionist may object that the level of description has been changed from the important to the trivial—from morphological to biochemical differences.

Dr Lewontin has not only played a large part in the development of theory but was among the first to answer the second question experimentally, in 1966, by showing that in wild populations of *Drosophila* there is probably genetic variation at the majority of loci specifying peptide chains; a finding subsequently confirmed in many species by other workers. This book derives from a series of lectures he gave in Columbia in 1969 and, consequently, perhaps suffers in structure. It is not as wide in scope as the title would suggest and concentrates too much on the evidence from gel-electrophoresis. The approach is historical and the first 100 pages are almost a grand build-up to the presentation of the answer to my second question. The theoretical framework is presented as an antithesis between two views. In the first of these —the 'classical' view—most of the genetic variation in populations is thought to be selectively neutral or harmful. Much evolutionary change may not have been adaptive but has resulted from the chance fixation of neutral alternatives. In the other view—the 'balance' view—it is held that variation is actively maintained in population by selective forces, although there would be disagreement about their exact nature, and that evolution proceeds by the active fixation of available alternatives at loci, with a gradual change of selection pressures over time. The necessary synthesis has not been achieved (though the author clearly leans scientifically and politically on the 'balance' side); Lewontin suggests that the profusion of facts which are now available for the 'theory machine' have merely led to "a great clashing of gears".

There are perhaps two main reasons for the present confusion. The first is that all wild populations have to cope with the wide diversity of environments, both in space and time, and we have neither an adequate description of these, from the point of view of the organism, nor an adequate theoretical treatment of their implications. The second is that differences in fitness between genetic alternatives may be so small (say of the order of 1% or less) that there is great difficulty in measuring them adequately. This is particularly true for the measurement of fertility. With *Drosophila* far too much work has been concentrated on the measurement of viability differences, which is relatively easy to do, even though recent evidence shows that fertility differences are much more important. In my view, the great merit of the book lies in its critical discussion of the present confusion and of the validity, both biological and statistical, of some of the evidence that other workers have put before us.

But we must remember that the loci that we observe with these techniques only refer to a small part of the DNA, the 'single-copy' set coding for proteins. The evolutionists have hardly faced up to the evidence now accumulating from the multiple-copy sequences, such as the satellite DNA or those sequences which code for ribosomal RNA. I suspect that the mechanisms that we shall have to invoke for the evolution of this part of the DNA will lie quite outside our present philosophy and outside the scope of this book. **Alan Robertson**

In honour of Lanczos

Studies in Numerical Analysis. (Papers in Honour of Cornelius Lanczos.) Edited by B. K. P. Scaife. Pp. xxii + 333. (Academic: London and New York, September 1974. Published for Royal Irish Academy.) £5.00; $13.00.

THIS Festschrift volume is published under the auspices of the Royal Irish Academy as a tribute to Cornelius Lanczos on the occasion of his 80th birthday (February 2, 1974). It is devoted almost exclusively to numerical analysis, since, although Lanczos made significant contributions to areas of theoretical physics, he is most widely known for his work in this particular field. Professor Lanczos died in Budapest on June 24, 1974 but, happily, he had lived to see this personal and affectionate tribute to him from many distinguished scholars.

Lanczos was educated in Hungary and worked first in Germany (1922–31), then in the United States (1931–54), and lastly in Eire (1954–74), and he was completely imbued and enthused with the traditional viewpoint of the complementary interaction between mathematics and physics, as exemplified by the Göttingen school of mathematics founded by David Hilbert. His work may be divided into two main areas: relativity theory and quantum theory (1922–38) and numerical analysis (1938–74), and in both of these he made important and lasting contributions to knowledge; his abiding interest lay with geometry and the nature of space and time. Lanczos was a prolific author of papers and books, which show clearly his viewpoint of the essential unity of mathematics and physics (the viewpoint, for example, of John von Neumann) and their cultural appeal and importance (as, for example, discussed by Morris Kline).
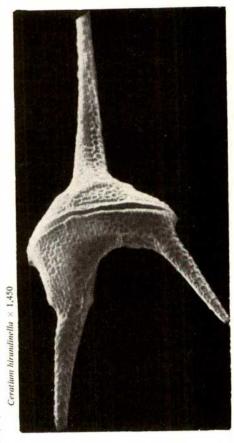
The Festschrift opens with a biographical note on Lanczos, supplemented by a list of publications by him (94 articles and eight books), and it continues with 19 contributed articles. Professor B. K. P. Scaife, who has charmingly dated his editor's foreword for Lanczos' birthday, has obviously sought, by the particular choice of contributers, to associate as far as possible current developments in numerical analysis with Lanczos's original ideas in that field. These include the application of Chebyshev polynomials in approximation theory and the development of the economisation (or telescoping) method and the tau method (1938); the fast-Fourier transform (1942); the first exact method for obtaining all the eigenvectors and eigenvalues of an arbitrary matrix (1951); a fundamental theorem on the decomposition of an arbitrary matrix (1958); a precision approximation to the Gamma function (1964); and Sigma factors for smoothing Gibbs's oscillations in Fourier series (1966).

In summary, in addition to articles of a specialist nature on topics in numerical analysis, there are three articles of a biographical nature and also a review article on conservation laws in Einstein's general theory of relativity. The book will appeal especially to those with interests in numerical analysis and also to those interested in the life and achievements of Cornelius Lanczos.

H. G. Hopkins

Red tide

Ceratium hirundinella × 1,450

Fossil and Living Dinoflagellates. By W. A. S. Sarjeant. Pp. vii + 182. (Academic: London and New York, December 1974.) £5.00; $13.00.

THE gulf called *Sinus arabicus* by the Romans was known to the Arabs themselves as the Red Sea, and it was under that name that it became known to the rest of the civilised world at least as long ago as the ninth century. The visually spectacular effects of the 'red tide' blooms of dinoflagellates have been recognised, therefore, for over 1,000 years, but not until the publication of this work has a book been devoted solely to these extraordinary organisms. Even if there were no other reason, that aspect alone must recommend Dr Sarjeant's volume, but the whole has the additional merits of clarity, readability and profuse, well chosen illustrations. The publishers maintain their high standards with good quality paper, clear printing and format, and strong and attractive binding.

Dr Sarjeant is a palynologist and micropalaeontologist; originally, he intended to deal only with the fossil dinoflagellates and their resting cysts ('hystrichospheres'), and it is with these that his strength lies. Appropriate techniques of sample preparation are outlined clearly and fully, thecate dinoflagellates and cysts are described morphologically, and a separate short chapter is devoted to the taxonomic problems which have resulted from nomenclature based separately upon thecae and cysts. A concise but thorough suprageneric classification of fossil dinoflagellates is appended, and the stratigraphic history of the group is summarised. The acritarchs are mentioned but not described in detail, and there are no illustrations or descriptions of the great majority of the genera and species listed in the stratigraphic section; the reader must turn to the references given by the author for that information. Recent attempts to achieve an objective terminology for the description of cyst walls are not discussed at all. Otherwise, the account is very comprehensive, and it is invaluable to have it (and such a valuable collection of reference lists) now so conveniently to hand.

The chapters dealing with biology, ecology (12 pp.) and palaeoecology (6 pp.) are much less thorough. The faculty possessed by some species to generate extraordinary levels of bioluminescence, with marked periodicity, still deserves further treatment—not only for its own sake, but also for the field work it has stimulated which is contributing greatly to our knowledge of the ecology of these organisms. Much detailed ecological work has been done in recent years, not least in the Red Sea itself, where dinoflagellates dominate primary production for most of the year. The important role played by dinoflagellates in food webs deserves more attention: symbiotic dinoflagellates (zooxanthellae) form a significant primary role in the productivity of coral reefs, and the motile swarms of the 'red tide' itself make their contribution to nutrition as well as to spectacular mass mortalities.

Dr Sarjeant's book will surely deserve a second edition on the merit of its micropalaeontology alone; if neontology and ecology could then be equally well covered, it would undoubtedly be a major contribution to synoptic studies in the natural sciences.

F. T. Banner

Landscape a l'americaine

The Origin of Landscapes: A Synthesis of Geomorphology. By H. F. Garner. Pp. xx + 734. (Oxford University Press: London and New York, October 1974.) £10.75.

THIS long and well-produced text is designed for use at US college level and presumably, therefore, for undergraduates in European universities. In it the author attempts to combine geology, geomorphology, climatology, botany and other branches of natural science in a synthesis of landscape study. The resultant theme seems to be halfway between physical geography in the European sense and physical geology in the American sense.

The presentation of the geomorphic subject matter is said by the author to be "at once new and not new" insofar as it emphasises the relationships of processes and treats any particular process in its several environments: for example, the effect of running water at the surface is discussed in humid, arid, and glacial contexts, and again with respect to alternating environmental conditions. The general theme reflects the genesis of landforms by associations with environmental systems and through the substitution of one system for another in time and space. Thus, the essential theoretical thread that binds together the otherwise detached concepts is the fact that most landscapes are the products of environmental sequence.

The main body of the text opens with a chapter on the concepts of geomorphic theory and the development of geomorphic ideas. The discussion is lively and stimulating but needs some revision. For example, the author thinks the peneplain was the ultimate landform in the Davisian cycle whereas it was penultimate; he considers that downstream increase in the velocity of rivers augments their erosive power whereas, presumably, the velocity is increased because of relative decrease in bed friction or erosional contact.

This introductory matter is strengthened by the succeeding chapter on geomorphic techniques which summarises modern advances. That is followed by a long account, forming nearly one-seventh of the book, on recent tectonic theories and findings, including plate tectonics, mid-oceanic ridges and sea floor spreading. The excellence and detail of this well-illustrated synopsis are not maintained in the next chapter which deals with "surficial geomorphic patterns" and contains *inter alia* a rather elementary account of the atmosphere and of the circulation of the oceans.

The chief superficial landscape systems are discussed. Humid, vegetated environments—the Davisian norm—are given far less textual space than are glaciation and glacial landforms, arid non-vegetated systems or alternating arid–humid environments. Coasts and mountains are treated separately as polygenetic landscapes, the former being the zone of contact between two distinct environments and the latter the areas within which internal uplift conflicts markedly with external erosion.

The closing chapters consider the application of geomorphological techniques to the theoretical reconstruction of ancient buried landscapes, and the nature of the various physical problems associated with alterations of the present day environment. The volume ends with a glossary of basal geomorphological terms which will be useful for beginners, and a comprehensive index that includes reference to the many striking illustrations, about 600 in all.

An attractive and lavish production, with many progressive ideas, this volume is a welcome and worthwhile addition to educational geomorphological literature. It is not easy, however, for a European to assess it with equanimity. The author thinks that Great Britain is "an island near the Gulf Stream" and in his index Europe receives 17 entries against 33 for Ecuador, 31 for Venezuela and 25 for Peru. Such a locational bias can be understood as the text is intended for American colleges; but less understandable are the frequent misuse—for depicting spatial distributions—of Mercator's cylindrical world projection (with each pole exaggerated in length 24,000 times to equal the equator), and the occasional textual error, such as Gibralter, duracrust, mollasse, and cannons (for canons of landscape evolution). Occasionally, too, the language probably becomes rather too-American or too askew to appeal to European taste. Is there arising an Old World–New World linguistic divide? We read that "coastal geomorphic systems . . . exist where the sea embraces the land, lingeringly, and often with what might well pass for passion"; that "the tectonics of plates is a fascinating jigsaw puzzle with many of the nobler attributes of a floating crap game". Whereas the pundits of the Oxford Press seem ignorant of the evils of Mercator, they are obviously experts at crap. **Robert P. Beckinsale**

Borers, foulers, sprats, and mangroves

The Biology of Estuaries and Coastal Waters. By E. J. Perkins. Pp. ix + 678. (Academic: London and New York, 1974.) £14.60; $37.35.
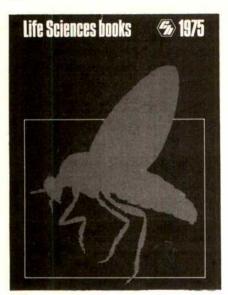
PERKINS' attempt to review comprehensively the biology of estuaries and coastal waters has resulted in a large book. The first fifth is introductory and covers the basic physico-chemical and dynamic characteristics of inshore waters; but the treatment is terse and fragmentary. No reader is likely to benefit from such short sections on tides and waves; and the hydrography of estuaries—so fundamental to what follows—surely merits thorough treatment.

The biota of the various habitats are described in the middle portion of the book, and environmental factors which limit distributions and/or vital activities are discussed. Microorganisms, plants and animals are covered, so most readers will find new information here. There are inconsistencies in approach: discussion is limited largely to estuarine plankton although rocky shores are mainly non-estuarine. And it seems a pity that the ecologically significant differences in the timing of primary production cycles in oceanic and neritic water masses could not have been mentioned. Rocky shore zonation is introduced principally by reference to one British locality but, as coverage (here and throughout the book) is by no means confined to the British Isles, a generalised Stephensonian scheme would have been more appropriate: the pages on Australian zone-formers may then have integrated better. The superficial approach can be appreciated when it is considered that, although sedimentary beach biota include salt marshes and mangroves the description of this group is restricted to 15 pages.

Even so, the author's interest in the interactions between man and the coastal environment is apparent, and that theme at least is expanded. The detrimental activities of borers and foulers are covered, as is the production of those organisms which man uses for food. Shellfish, except for tropical prawns, are treated fairly fully; but fish, as elsewhere in the book, receive scant attention. Thus the sprat, with an annual catch of up to 100,000 tons in the estuaries and embayments of the North Sea, gets only an incidental mention, and the importance of estuaries as herring and sprat nursery areas is omitted.

The chapter on pollution is somewhat philosophical, but waste disposal and management are both discussed. Prominence is given to the high standards laid down by the United States Federal Water Quality Administration, which contrast with the low standards (except for radioactive waste disposal) acceptable in the UK.

By opting for breadth the author opens himself to criticism for the brevity with which many topics are treated, especially in those fields in

which he seems not entirely at home. But the compilation is impressive and uses a vast literature. Unfortunately, though, the bibliography is presented chapter by chapter in the sequence in which they are mentioned, so the location of references is an exasperating business. Mistakes and typographical errors are few and the book is well produced; but the price is far beyond the means of many of those who would wish to buy it.   **J. S. Ryland**

Life

Life—The Unfinished Experiment. By S. E. Luria. Pp. 167. (Scribner's Sons: New York, June 1973.) $7.95.

SALVADOR LURIA is well known for his work in virology and molecular genetics. In 1969 he shared the Nobel Prize in Physiology with Delbrück and Hershey. His aim in the present book is to explain modern biology and its social relevance to the general reader.

The scope of Luria's book is superficially the same as Monod's *Chance and Necessity* but there are significant differences. The scientific parts of *Chance and Necessity* were too advanced for the general reader. Luria has attempted a more elementary account. He also deals less with metaphysical issues than Monod, and his social and ethical views are differently slanted. I believe that Luria has succeeded in producing a valuable and readable book, and that it could be usefully incorporated into science courses in schools and generalist courses in universities; and that it would be of interest to laymen and to some professional biosciences.

Luria emphasises molecular aspects of biology, but not to the exclusion of organismic aspects. He builds up a masterly picture of biology, bringing out key concepts in lively, non-technical ways. Starting with an introduction to evolution theory, he goes on to genes, cells, bioenergetics, form and function in higher organisms, and biogenesis. He concludes by discussing social and philosophical issues connected with biology. All this he compresses into only 150 pages, without losing readability, by pruning out unnecessary detail and dealing only briefly with many of his points. Those with little previous biological knowledge will need supervision or complementary reading to appreciate the more subtle points. An apposite reading list is appended to the text.

Luria's underlying theme is "the dualism of the material nature of life's programme on the one hand and the historical nature of biological evolution on the other hand". In his chapter on evolution theory he first discusses the impact the theory has had on man's image of himself and of nature, and

then goes on to consider the scope and limitations of the theory. He emphasises the mutual interaction of individual and environment, and accepts the 'genetic assimilation' view that selection acts on phenotypes rather than on genotypes. In his chapters on heredity, he introduces the subject in a striking way by comparing modern genetics to the great generalisations of physics. At the same time, he points out the uniquely high degree of order in biological phenomena and its historically-derived nature. In each of the succeeding chapters, he also presents the subjects in stimulating and illuminating ways. He does not try to gloss over difficulties and uncertainties.

In the penultimate chapter, Luria discusses issues of medicine, population and genetic engineering. He considers the humane regulation of population growth to be of overriding importance, but recognises the great social and political problems involved. He discusses possible biological consequences of population control and maintains that eugenics programmes (apart from those meant to reduce serious genetic disorders) should be avoided for both social and biological reasons. Though recognising the great technical obstacles to direct genetic intervention in man (such as genetic engineering and cloning), he considers that "in science, once a task has been clearly defined, its accomplishment is generally only a matter of time", and that some genetic intervention may be in medical use within 30 years. And he explores the social and ethical problems that this would raise in our "competitive, caste-ridden, power-dominated society"

In the final chapter, Luria gives a stimulating glimpse into some philosophical issues relating to biology, such as language and brain structure, nativism and learning, biological and cultural evolution, determinism and free will, values, and existential dilemmas. He emphasises the uniqueness of every human individual. He locates the central dilemma of life science as the relations of human purpose, values and will to the fortuitous nature of the evolutionary process. He considers the human brain to have arisen through a process in which "evolution has dialectically surpassed and denied its own past history". Inevitably, various controversial points arise. Among these, I believe that Luria's treatment of existential problems in terms of natural selection is weak; and I would like to see the physicalist position—which he presupposed throughout—explicated and examined in relation to possible counter-evidence (from, for example, parapsychology). In spite of such criticism, my impression is of a well balanced, humanly sensitive, and penetrating world-view.   **Robin Monro**

obituary

Roderick O Redman, FRS, a leading British optical astronomer, has died at the age of 69

Professor Redman studied mathematics at St John's, Cambridge, gaining his Ph D in 1929 At Cambridge, he was greatly influenced by Sir Arthur Eddington, Plumian Professor of Astronomy, and took up his first appointment with the Dominion Astrophysical Observatory at Victoria, British Columbia He returned to the Cambridge Observatory in 1931 to become its Assistant Director and was elected a fellow of St John's in 1932 In 1937, he became Chief Assistant at the Radcliffe Observatory in Pretoria, South Africa, where he started his researches on astronomical photometry While in South Africa, he made an expedition to the total solar eclipse of 1940, from which he obtained high dispersion chromospheric spectra superior to those obtained previously Returning to Cambridge in 1947 as Professor of Astrophysics, he fully integrated the Solar Physics Observatory into a new unit, called the Cambridge Observatories Under his direction, they rapidly recovered from the difficulties of the war years His enthusiasm and warmth were his major assets in re-equipping the observatory with good modern instruments Redman led an equally successful expedition to Khartum in 1952 and served as President of the Royal Astronomical Society from 1959–61 Much of his time before and after his retirement was devoted to the planning and construction of the 150 inch Anglo-Australian Telescope and the success of this instrument owes much to his enthusiasm After his retirement, he also took part in the planning of the Northern Hemisphere Observatory

Anatolii Arkad'evich Blagonravov, academician and one of the leading figures in the Soviet space programme, died on February 4 at the age of 90

Blagonravov, a Lieutenant-General of Artillery, and a full Member of the Academy of Sciences of the USSR since 1953, became attached to the Soviet space programme during the 1950s as a rocket designer In 1959, he addressed the press conference held in connection with the launching of Sputnik 1 From 1960 onwards, he published a number of works on space research, including *Physics of Cosmic Space* (1962) He was an early member of the Pugwash movement, Deputy Representative of the Soviet Union at the UN Committee for the Peaceful Use of Space, and Chairman of the Commission of the Academy of Sciences of the USSR on Space Research and Utilisation He was a full member of the Czechoslovak Academy of Sciences, and a Member of the International Academy of Astronautics, and held a number of Soviet medals and awards, including the Order of Lenin (five times), the Order of the Red Banner of Labour (three times) and Hero of Socialist Labour

Clarence Ray Carpenter, the psychologist and anthropologist, died in Athens, Georgia, on March 1 at the age of 69

Dr Carpenter studied monkeys and apes for the clues they gave to human behaviour and began his studies on the social life of monkeys in Central America in the 1930s In 1937, he was a leader of the Asiatic Primate Expedition to Thailand and Sumatra, and studied gibbons in Siam from the point of view of the evolutionary background of human society In 1939, he travelled from India with 500 macaques that he resettled on Santiago Island, off the east coast of Puerto Rico, observing them set up home In 1960, Dr Carpenter took a census of the howler monkeys of Barro Colorado Island, Canal Zone, hoping that a study of their ways would serve as a conceptual bridge between the realms of lower animals and man From 1940–69, he was on the staff of Pennsylvania State University and became emeritus professor of psychology and anthropology, directing the use of closed-circuit television for educational purposes there As a leader in promoting educational film and television techniques, he served on the Commission on Instructional Technology and on the Advisory Committee of the US Office of Education, and was a past-president of the Joint Council of Educational Communications and the Association for Higher Education Dr Carpenter was a former editor of *Behaviour* and of the *Journal of Evolution*

announcements

Awards

Her Majesty the Queen has approved the grant of a **Royal Charter** to the **Institution of Metallurgists** at a meeting of the Privy Council **Dr W. E. Duckworth** will be succeeded in May as president by **Sir Montague Finniston, FRS.**

The Zoological Society of London has made the following awards **Scientific Medal** to **P F. Baker** (ionic transport across cell membranes) and **H. Kruuk** (behaviour and ecology of gulls and carnivores), **Stamford Raffles Award** to **A. E. Ellis** (study of molluscs), **T. H Huxley Award** to **I G. Priede** (circulation during swimming in trout), **Silver Medal** to **E. Hosking** (animal photography and educational zoology), **Frink Medal** to **J. Z. Young, FRS** (original contributions to wider implications of Zoology)

A. R Ubbelohde, CBE, FRS, has been awarded the **George Skakol Memorial Award** by the American Carbon Society for contributions to the understanding of the intercalation of graphite by elements and compounds, and of the electrical and thermal properties of graphite

The Society for Analytical Chemistry has awarded its **Gold Medal** to **R. L. Mitchell** for contributions to emission spectroscopy for trace elements

International meetings

April 15, **Biochemistry of Blue-Green Bacteria,** Aberystwyth (Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP, UK)

April 16, **Mechanisms of Biocidal and Biostatic Activity,** Aberystwyth (Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP, UK)

April 30–May 3, **Protides of Biological Fluids,** Brugge, Belgium (XXIIIrd Colloquium on Protides of Biological Fluids, Simon Stevin Instituut, Jerusalemstraat 34, B-8,000 Brugge, Belgium)

May 8–12, **Flint**, Maastricht (Mr J H M Nillesen, mesweg 19, Eys-Wittem, The Netherlands)

May 13-16, **International Radiation Protection**, Amsterdam (C E Rasmussen, Interuniversitair Reactor Institute, Berlageweg 15, Delft, Netherlands)

May 14-15, **Ionic Polymers**, Brunel University (Mrs R Saunders, Industrial Liaison Bureau, Brunel University, Kingston Lane, Uxbridge UB8 3PH, Middlesex, UK)

May 15-16, **Ethical and Social Questions Posed by 'Engineering' the Human Genome**, New York (Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021)

May 25-28, **Genetic Hazards to Man from Environmental Agents**, Ottawa (Mrs J Renaud, Room 1-5, Health Protection Building, Health and Welfare Canada, Tunney's Pasture, Ottawa, K1A 0L2, Ontario, Canada)

May 26-30, **Prostaglandins**, Florence (Dr G C Folco, Institute of Pharmacology and Pharmacognosy, University of Milan, Via Andrea del Sarto, 21, 20129 Milan, Italy)

May 28-30, **Asthma and Allergy**, Denver, Colorado (Elliott Middleton, Jr, MD, National Asthma Center, 1999 Julian Street, Denver, Colorado 80204)

May 30-31, **Myasthenia gravis**, New York (Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021)

June, **Protein Structure and Evolution**, Prague (Dr Z Deyl, Czechoslovak Academy of Sciences, Institute of Physiology, Praha 4-KRC, Budejovická 1083, Czechoslovakia)

June 2–5, **Information Transfer in Eukaryotic Cells and Recognition and Responses in Lymphocytes**, Montreal (W Fred Hink, Ohio State University, College of Biological Sciences, Department of Entomology, 1735 Neil Avenue, Columbus, Ohio 43210)

June 2-6, **Atomic Masses and Fundamental Constants**, Paris (AMCO-5 Secretariat, Institut d'Electronique Fondamentale, Bât 220, Université Paris-Sud, F-91405 Orsay, France)

June 2-6, **Peptides**, New York (Conference Department, New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021)

June 4–6, **Identification and Role of Cellular Receptor Sites**, Baltimore (Edward G Bassett, Ph D , Symposium Coordinator, Miles Laboratories, Inc , Elkhart, IN 46514, USA)

June 7–8, **Biological Monitoring of Water Quality**, Helsinki (Jean-Louis Gaudet, UN Food and Agriculture Organisation, Via delle Terme di Caracalla, 00100-Rome, Italy)

June 9–11, **CO₂ Metabolism and Productivity of Plants**, Madison (Marcia Molldrem, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706)

June 15–18, **Plants and Cancer**, Baltimore (Dr Ralph N Blomster, Chairman, Department of Pharmacognosy, School of Pharmacy, University of Maryland, 636 W Lombard St , Baltimore, Maryland 21201)

Person to Person

Golfers. Edinburgh Medical Technicians Golfing Society are organising a 50th anniversary Open Golf Tournament at Broomieknowe Golf Club Midlothian on July 8 Prizes to the value of £200 Open to all Medical and Biological Sciences personnel who hold a national handicap Entry forms from David Veitch, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

New physical and astronomical theory Scientist wishes to start international organisation to develop a physical and astronomical theory without Einstein's relativity theory, without the quantum theory and without a postulate or principle which is not proved by a positive experiment within ten years Those wishing to cooperate, contact Ir P A v Deinse, Adm v Gentstr 9, Utrecht The Netherlands

Weight-driven laboratory clock. Wanted, on loan or hire, one of those 'retired' regulator clocks, for a programme of experimental and theoretical work on pendulum and escapement errors Collection and transport arranged, assurance (if required) against damage or permanent alteration (Dr D M A Mercer, Department of Physics, University of Southampton, SO9 5NH, UK)

Reports and publications

Great Britain

Imperial College of Science and Technology Sixty-seventh Annual Report of the Governing Body, 1973/1974 Pp vi + 81 Annual Accounts, 1973/1974 Pp 28 (London Imperial College of Science and Technology, University of London, 1975) [241

Psychoenergetic Systems Vol 1, Part 1, December 1974 Pp 1–48 Subscription Rates (per volume of four issues, postpaid) Great Britain Individuals who warrant that the journal is for their own personal use and who order direct from the publishers, £6, Libraries, Institutions and others £22 75 USA/Elsewhere Individual subscribers $19 50/£8 50 Librarian, Institutions, and others $58/£25 (London and New York Gordon and Breach, Science Publishers, 1974) [241

Neuropathology and Applied Neurobiology, Vol 1, No 1 January 1975 Edited by J B Cavanagh (Journal of the British Neuropathological Society) Pp 1–110 Published Quarterly Annual subscription £14, USA and Canada $47 50 Single issues £4, USA and Canada $13 50 plus postage (Oxford and London Blackwell Scientific Publications, 1975) [271

Other countries

Environmental Impact Assessment Principles and Procedures Edited by R E Munn (SCOPE Workshop on Impact Studies in the Environment (WISE) co-sponsored by United National Environmental Program (UNEP), Environment Canada and UNESCO) Pp 160 (Toronto International Council of Scientific Unions Scientific Committee on Problems of the Environment, 1975) [131

New Zealand Meteorological Service Misc Pub No 109 Meteorological Observations for 1973—Stations in New Zealand and Outlying Islands, including the Cook Group, Tokelau Islands, Niue Island and Western Samoa Pp 108 $1 50 Misc Pub No 110 Rainfall Observations for 1973—Stations in New Zealand and Outlying Islands, including the Cook Group, Tokelau Islands, Nieu Island and Western Samoa Pp 74 $1 (Wellington N Z Meteorological Service, 1973) [131

United States Department of the Interior Geological Survey Professional Paper 828 Fuller's Earth and Other Industrial Mineral Resources of the Meigs-Attapulgus-Quincy District, Georgia and Florida By Sam H Patterson Pp 45 + 2 plates (Washington, DC Government Printing Office, 1974) $2 40

Canada Department of Energy, Mines and Resources Geologica Survey of Canada Paper 74-20 Reconnaissance Glacial Geology, Northeastern Baffin Island By D A Hodgson and G M Haselton Pp 10 $2 Paper 74-25 The Geomorphology of the Swan Hills Area Alberta By Denis A St Onge Pp 6 $2 Paper 74-61 Stratigraphy of the Amoco IOE A-1 Puffin, B-90 Well Grand Banks of Newfoundland By W A M Jenkins P Ascoli, L F Jansa and G L Williams Pp 12 $2 Paper 74-58 List of Translations in the Library of the Geological Survey of Canada, No 3 Compiled by C Joanna Christensen Pp iv + 43 $2 (Ottawa Information Canada, 1974) [131

United States Department of the Interior Geological Survey Professional Paper 844 Geophysical Investigations of the Pensacola Mountains and Adjacent Glacierized Areas of Antarctica By John C Behrendt, John R Henderson Laurent Meister and William L Rambo Pp iii + 28 + 2 plates (Washington, DC Government Printing Office, 1974) $2 05 [151

Norsk Polarinstitutt Skrifter Nr 161 The Billefjorden Fault Zone, Spitsbergen—The Long History of a Major Tectonic Lineament By W B Harland, J L Cutbill P F Friend, D J Cobbett, D W Holliday, P I Maton, J R Parker and R H Wallis Pp 72 (Oslo Norsk Polarinstitutt, 1974) [151

United States Department of Agriculture Index-Catalogue of Medical and Veterinary Zoology Special Publication No 3 Ticks and Tickborne Diseases II Hosts Part 1 A–F Pp vi + 1–490 Part 2 G–P Pp iv + 491 –976 Part 3 Q–Z Pp vi + 977–1268 By Mildred A Doss, Marion M Farr, Katharine F Roach and George Anastos (Washington, DC US Government Printing Office 1974) $12 05 per set of three parts Sold in sets only

Seismic Masking of an Underground Nuclear Explosion—Final Technical Report By Lawrence D Porter prepared for Air Force Office of Scientific Research, Arlington, Virginia) Pp xx + 110 (Pasadena, California Jet Propulsion Laboratory California Institute of Technology, 4800 Oak Grove Drive, 1974) [171

Smithsonian Contributions to Zoology, No 180 Clearwing Moths of Australia and New Zealand (Lepidoptera Sesiidae) By W Donald Duckworth and Thomas D Eichlin Pp iii + 45 (Washington, DC Smithsonian Institution Press, 1974) For sale by US Government Printing Office) $1 15 [171

Canada Department of Energy, Mines and Resources Geological Survey of Canada Paper 75-1, Part A Report of Activities, April to October 1974 Pp x + 602 (Ottawa Information Canada, 1975) $5 [171

International Atomic Energy Agency, Vienna Technical Reports Series No 158 Recommended Instrumentation for Uranium and Thorium Exploration Pp 93 (Vienna IAEA, London HMSO, 1974) 92 schillings £2 10 $5 [201

National Research Council of Canada Associate Committee on Scientific Criteria for Environmenta Quality—Subcommittee on Pesticides and Related Compounds Subcommittee Report No 2 Chlordane—Its Effects on Canadian Ecosystems and Its Chemistry Pp 189 (Ottawa Publications, NRC 1974) $1 [20]

Australia Commonwealth Scientific and Industria Research Organization The Division of Soils Biennia Report, 1972/1973 Pp 140 (Melbourne CSIRO 1974) [211

nature

Concentrate on fundamentals

"WE are here today", said Mr Ian Lloyd as he opened the Science Sub-Committee hearings on the needs of scientific research in British Universities, "to look into the funding of scientific research from all sources, public and private The University Grants Committee, the Research Councils, charities and foundations—we shall investigate the distribution of funding amongst them We shall also look at the relation of research to teaching, and the implication of changes in funding on research " To hear the six witnesses, there were three MPs, four pressmen and one member of the general public Hardly an auspicious beginning to a penetrating survey

On display were representatives from two of the newer and more technologically-oriented universities—City and Brunel It was difficult to discern any very clear threads from their presentations There was a certain amount of special pleading for things like tenured technicians, more travel money and less accounting duties, but the array of professors lost a major opportunity to put sharply in focus the plight of universities Instead they indulged in a rambling dialogue in which important points were left unmentioned or given inadequate emphasis Out of it all emerged a picture of two universities whose professors have slight chips on their shoulders about status and have a general desire to do something about the Science Research Council, but no very clear idea what they would do

The most worrying impression left was that whilst the educational system faced crisis and some departments are denuded of students, there is no serious thought given to a strategy for universities or any well marked-out policy options which are being discussed in common-rooms If universities themselves cannot present compelling evidence that they are thinking carefully about policy, they must expect that policy will be decided for them

The primary need of scientific research is a continuous flow of highly intelligent manpower, and it is to this problem that the committee should pay greatest attention, for if the numbers coming into science at the undergraduate level continue to decline, then the *raison d'être* for many university staff disappears and any call for new facilities and equipment or even for the long-term financial commitments that every Vice-Chancellor dreams of landing is bound to be muted Unfortunately, few in the university environment have yet come up with much original thinking either on how to reverse the decline or what to do if the decline proves irreversible There are, of course, various crumbs of comfort, up to the present the loss in recruitment has been mainly of the less qualified, and up to the present it has been possible to fill classes up with overseas students But both of these situations could change within a year or two for the worse

Although a City representative did touch on the "disastrous shortfall" in native-born applicants for graduate studentships, he did not pursue any line of thought on reasons why or remedies considered—nor, one suspects, will many of those who come to give evidence It is unthinkable that a business which saw a steady decline in demand for its services would not go to great lengths to learn about the trend and try to respond to it, the committee would do well to ask some plain questions of future witnesses on their understanding of the decline in recruitment to science—maybe to the extent of asking whether the trend towards producing 44 equal universities (and even moving the polytechnics up to a level footing) is an intelligent response to a situation in which the science departments of half these universities are gasping for students

Future witnesses might also be at some pains to spell out their views on the purpose of a university Not unnaturally, in times like these the committee is pursuing rather doggedly the question of science in the national interest and the need for better industry/university links Science for the country is a particularly tricky issue and needs some careful prior thought If the laboratories really are full of overseas students, and if the international corporation, capable of exporting technology at will, increasingly dominates science-based industry, then talk of national interest is bound to become of diminishing relevance

Certainly the international context of science—even the European dimension—is a difficult idea to get through to politicians who obviously want to see returns for taxpayers' money And yet to fail to make the broader scene clear would be to run the risk that university science departments be forced to pay too much attention to presumed national needs—and too little to the advancement of learning □

international news

CONTINUING its investigation of the way in which energy is being conserved in the UK, and at the same time reinforcing its disbelief at what it sees as a "surfeit of inconclusive thinking" on the part of the government, the Energy Resources Subcommittee of the Select Committee on Science and Technology last week turned to the chemical industry, represented by three members of the Chemical Industries Association (CIA) Limited

The subcommittee was clearly anxious to hear firm advice from the CIA about what sort of measures the government should be taking to see that energy is not wasted In the event, what it got were some general comments about price restraint and taxation, but few specific recommendations

The CIA did say, however, that the chemical industry in the UK expects to reduce its energy requirements per unit of output by about 10% by 1980, principally by good energy 'housekeeping'—maintaining a watchful eye on the amount of lighting used, unnecessary leakages of steam, the quality of insulation and lagging, and so on Capital investment also helps, but usually only in situations where the increased cost of energy has made it economically sensible to replace equipment before it is worn out As the association pointed out, although energy costs to the chemical industry have gone up by about 80% between 1973 and 1975, capital costs are not far behind with an increase of some 55%

The CIA emphasised, however, that improvements in chemical manufacturing techniques could make for extensive energy savings Some 4 million MW h of the 19 million MW h of electricity

British energy users' views

by Roger Woodham and Eleanor Lawrence

used by the chemical industry in a year —equivalent to about 460 MW out of 2,200 MW on a continuous basis—goes in the manufacture of chlorine alone, the CIA stated by way of an example, and the efficiency of that process has been increased in recent years by some 5% as metal anodes have come to replace carbon ones Quite a respectable saving of energy

Mr G G Harrison, of ICI, also confirmed the need for courses in energy awareness for people entering and working in the chemical industry

● Following the CIA, the Energy Subcommittee took evidence this week from another massive energy user, the British Steel Corporation (BSC) With an annual total fuel bill of £600 million and a 10% share of Britain's total energy requirements, the steel industry is obviously in a position to affect the government's energy-saving plans considerably

The main burden of BSC's argument was that modernisation of its plant and processes is essential if any further saving in energy consumption is to be made If modernisation could go ahead quickly, the BSC reckoned on reducing the energy costs per tonne of steel produced by 10% by 1978

Unlike many industries where energy costs have previously been only a few percent of total basic costs, British Steel has always been aware of the

possibilities of a more efficient use energy Since 1967 it has made a red tion of 13% in its energy requiremei but the 10% reduction envisaged to 1978 would represent a mi stepped-up effort Asked about possible shortage of qualified fuel eng eers, BSC's Director of Research i Development admitted ruefully that energy crisis and the growing need fuel engineers had meant a mass turnover in BSC's own engineeri force as it was one of the few sourc of trained, experienced fuel engine in the country He envisaged that much more extensive in-house traini scheme would be necessary in t future

A long term possibility for minim ing energy costs for the steel industry to harness the heat-requiring reactio (basically the reduction of iron or directly to the process heat fro nuclear reactors without the inte mediate generation of electricity U fortunately for the steel industry Britain, the only reactor capable reaching the required temperatures around 1,000 °C is the high temper ture reactor, which is not exactly high priority in Britain's nuclear r search programme The Select Commi tee appeared slightly pained to lear that we should have to import this typ of reactor technology from the USA Japan or Germany in about 15–20 yeai when the link-up has been sufficientl developed But as BSC gently pointe out, it was hardly fair to expect commercial reactor to be develope without a market for electricity genera tion This market has now bee cornered by the SGHWR, the Seleç Committee's own choice

IN a further step forward in the British government's efforts to cut defence expenditure from 5 8% of GNP in 1974 to 4 5% in the late 1970s, the Statement on the Defence Estimates, 1975 (Cmnd 5976, HMSO, £1 22) announces more explicitly the targets to be aimed at Previous plans were for expenditure (at 1974 prices) to rise from about £4,000 million for 1975–76 to £4,300 million in 1978–79 and £4,450 million in the 1980s Now the aim is to cut £300 million a year off the budget in the next few years and eventually, in the 1980s, to be paying only £3,790 million for defence

Research and development expendi-

ture is also to be cut fairly heavily Throughout the 1960s the proportion of the defence budget devoted to it declined steadily from 15 5% (1961) to 9% (1970) Since then there has been a steady rise back to 12% and the plans were for it to stay at that figure until 1978–79 and then decline to 10% by the mid-1980s Revised plans call for no substantial change in these percentages, but in absolute terms £440 million (1974 prices) less will be spent in the coming decade on research and development Research accounts for about one-sixth of the expenditure, and of that sixth about one-fifth is called 'general or basic' research One-

third of expenditure on research and development goes to aircraft, another third to guided weapons and electronics 38,000 civilians and 1,500 military personnel work in the research and development field

Part of the cut in expenditure that will be called for should come from the rationalisation process now going ahead in research and development establishments The plans are for four 'systems' establishments for the four environments sea, land, air and underwater, and a number of 'technology' establishments dealing with service-wide requirements such as communications or propulsion

THE final chapter is yet to be written in that 50-year squabble between the Administration and Congress over whether or not the United States should ratify the Geneva Protocol, which prohibits first use in war of chemical weapons Although the Senate voted to ratify the Protocol in December last year and President Ford signed it on January 22, legal doubts about the Administration's interpretation of its terms have delayed the final stage in ratification—the articles have not yet been deposited with the government of France, which means that the United States is still not a formal party to the treaty

The problem, according to sources in the Administration, involves the question of whether or not herbicides and tear gases are covered by the Protocol When President Nixon resubmitted the treaty to the Senate for its approval in 1970, he did so with the understanding that a formal reservation would be written into the ratification, stating that the United States government believes that herbicides and tear gases are not included within the terms of the protocol The Senate Foreign Relations Committee refused to accept that understanding, however, and an impasse developed until late last year, when a compromise was worked out between the Ford Administration and Senator William Fulbright, the chairman of the Foreign Relations Committee It enabled the treaty to be approved unanimously by the Senate

The compromise, in short, allowed the Administration to retain its belief that herbicides and tear gases are not covered by the Protocol, but no formal reservation to that effect would be written into the ratification Instead, President Ford announced that he would issue an executive order setting out a 'national policy' that the United States would never be the first to use those agents in war, except in four minor instances (such as clearing undergrowth around military bases and controlling rioting prisoners of war)
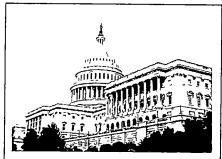
The executive order has, however, never been issued because it is under legal review in the Justice Department, and until that review is completed, the articles will not be deposited with the French government The review was initiated by President Ford's legal counsel, Philip Areeda, because of doubts about the legal status of such a 'national policy' Since the Justice Department has little experience in such international matters, the review is taking considerable time

One Administration official last week described the affair as simply a "bureaucratic snafu" which would soon be resolved He pointed out that the

Japanese government has adopted a similar interpretation of the Protocol, and its ratification of the treaty has not been challenged on legal grounds

Nevertheless, it should be noted that the United Nations adopted a resolution in 1969 stating that the protocol covers use in war of all chemical agents

● Meanwhile, the Department of Defense has, as expected, renewed its

Washington seen
by Colin Norman

request for Congress to provide funds to allow production of binary nerve gas weapons to begin Last year, Congress refused to provide $5 8 million for production of binaries, chiefly on the grounds that if the United States develops a new generation of nerve gas weapons, chemical disarmament talks now taking place in Geneva would be torpedoed Undaunted, however, the Department of Defence is now asking for $8 8 million for binary production

It is considered unlikely that Congress will approve the request, because the arguments raised against binaries last year will be just as strong this year Already, Mr Richard Ottinger, a liberal Democrat from New York, has introduced a resolution barring production of binaries and the campaign against the weapons is likely to be stepped up in the next few weeks

● There are indications that the Food and Drug Administration (FDA) may partially lift its controversial ban on cyclamates in the next year or so If so, the agency will bring down almost as much criticism as it encountered when it abruptly removed the sweetener from the market in 1969, following reports that it causes bladder cancer when fed in high doses to rats

The latest development in the cyclamate saga occurred last month, when the FDA informed Abbott Laboratories, the manufacturer of cyclamates, that it would ask the National Cancer Institute to determine whether or not the sweetener is a carcinogen The move represents a considerable retreat from the FDA's contention, announced in September last year, that insufficient

new data are available to justify reconsidering the ban

According to a letter sent to Abbott by Richard J Ronk, Director of the FDA's Division of Food and Color Additives, the question of whether or not cyclamates are carcinogenic "remains a difficult one to resolve"

Although he noted that some FDA scientists believe that there is sufficient evidence to conclude that cyclamates do cause cancer in rats, Ronk suggested that "it is the apparent opinion of the oncological community of the world that cyclamates when tested in accordance with appropriate protocols are not carcinogenic"

On the day that the letter was sent to Abbott, FDA Administrator Alexander Schmidt asked the National Cancer Institute to set up a panel of cancer specialists "with impeccable credentials" to review the evidence on the carcinogenicity of cyclamates as soon as possible The outcome of the cancer institute's review is crucial because a provision in the food and drug laws, known as the Delaney Amendment, forbids use of any food additive which is found to raise cancer in test animals Unless the review comes up with the conclusion that cyclamates are not carcinogenic, there is therefore no chance that the sweetener will be brought back on the market

But even if the National Cancer Institute gives cyclamates a clean bill of health as far as carcinogenicity is concerned, there are other doubts about the safety of the sweetener The FDA's letter to Abbott notes, for example, that tests have shown that cyclohexylamine—a metabolic product of cyclamates—causes softening of the testes when fed to rats Because of that, the letter hints that if the sweetener is allowed back on the market, the FDA would probably propose regulations to regulate intake to about 0 5 g per day

Although the tone of the FDA's letter seems to indicate that the agency believes that cyclamates are not carcinogenic, a final decision is not likely to be made for at least a year If the agency does lift its ban on cyclamates, a flood of complaints can be anticipated from Congress and from consumer groups, and the FDA is therefore trying to tread cautiously

● Congress has, as expected, rejected President Ford's suggestion that $351 million be cut from this year's budget for the National Institutes of Health Both the House and the Senate have passed bills directing Ford to spend all the money appropriated for this fiscal year—which now has only three months left to run—and so, barring last ditch delaying tactics by the Administration, the money will now be made available

Little progress for Indian education

from Narender K Sehgal, Jullundur

EDUCATION is a subject that has nearly always been topical in independent India—probably because, in spite of all the discussion and debate over it, so very little has been done during the past quarter of a century At present two-thirds of all Indians are illiterate Yet large numbers of the educated, and the highly educated are unemployed, not to mention the multitudes of the uneducated unemployed and those who are underemployed Such anomalies abound there is an acute shortage of doctors in rural India but at the same time not only are thousands of Indian doctors working abroad, there is also widespread unemployment among medical graduates in the country, India badly needs middle-level skilled professionals and other types of trained worker suitable for rural areas—the kind our system is just not turning out It appears that the Indian nation spends countless crores in educating and training individuals who only seem of value in foreign lands

These facts speak volumes about education in India, among other things The system of education—a legacy from the bygone colonial era—has been a constant target for criticism And understandably so, because the present system differs little in content and character from the one the English rulers had devised with a set of aims and objectives of their own—quite different, one would think, from those which might have most suited a free nation

In view of the general situation at the time of independence, the leadership thought it fit to maintain the *status quo* in education, as in many other areas, and to let the drastic changes wait till normality had returned But this turned out to be a blunder, because as time went by those who took charge of education, perhaps incapacitated by their own background and training, did little or nothing to devise a new system or to modify and improve the existing one As the Prime Minister, Shrimati Indira Gandhi, admitted as late as last year "One of the biggest mistakes we made when we gained independence was not to have overhauled thoroughly our educational system and structure We are paying for it now"

All that the government has been able to do in this direction till now is to appoint committees and commissions to go into various aspects of the system and structure of education These bodies have produced tonnes and tonnes of words in the form of voluminous reports which are gathering

dust on shelves in the offices of bureaucrats in the Ministry of Education and elsewhere One would like to believe that, armed with all these investigative and in-depth reports from eminent and learned men, the government would by now be quite clear about what needs to be done and how This, however, is not the case Confusion and uncertainty mark statements made from time to time by those in authority The education policy of the government of India seems to be unclear and lacking in definite direction

The Prime Minister herself has not helped matters by sounding inconsistent in her numerous speeches on the subject, and sometimes at variance with the approach educational planners in her own government would like to adopt On more than one occasion she has explicitly agreed with the widely held view that the present system of education must be drastically changed, or even discarded altogether, as in her own words 'it has little contact with life in the country   It belongs not only to another civilisation, it belongs to another century And we need a minor or even perhaps a major revolution   " On another occasion, not too long ago, she chose to caution educational planners against policies and drastic measures which would cause major 'dislocations' in the existing educational process As recently as February 1, while speaking at the Silver Jubilee celebrations of the National Chemical Laboratory in Poona, the Prime Minister said   "With all the known shortcomings of our educational system and of our organisation of science, the fact remains that India pro-

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SHEIK Zayed Bin Sultan Nahayam, ruler of Abu Dhabi, has a good line in greenhouses They are soon to cover a 15-acre site in the desert in an attempt to start the first commercial market garden in the Gulf States The greenhouses will be the plastic-dome type and the irrigation supply will be from water reservoirs that have been discovered under the desert by scientists from the French national oil company, CSP

The great problem with desert irrigation is, of course, evaporation This will be overcome by a recently discovered system in which hose pipes, punctured with small holes of calculated size and spacing, are laid out along the rows of plants Water is thereby delivered at the optimum site and rate to minimise waste

And who discovered the irrigation system? According to Israeli scientists it was devised at the Weizmann Institute (where it can be seen on the campus flower beds) Will Sheik Zayed give credit where it's due?

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duces exceptionally gifted scientists and an impressive number of highly competent scholars "

To cope with mounting unemployment among the educated youth, planners in the Central Advisory Board of Education (CABE) would like to adopt a strategy which stresses the vocational aspects of the educational process This, besides enhancing chances of employment and self-employment through inducements like financial and technical assistance from various governmental and other agencies, will also help ease pressure on enrolment in institutions of higher learning—in line with another CABE proposal which seeks to place drastic restrictions on expansion in higher education Again, Shrimati Gandhi in her Poona speech last month observed "Many student groups come to see me and often argue that our educational system should be given a vocational orientation   But training a person for a job is not the sole or the highest purpose of education, nor is it adequate for the longer run That purpose is to train better human beings who can give more to life and be capable of deriving more from life "

In regard to curbs on expansion in college and university enrolments (which have increased by 800% in the past 20 years), the Prime Minister cautions that it must not in any way result in limiting heightened expectations of those in the backward classes, and that such curbs on expansion of higher education must also be accompanied by concrete steps to bring about a change in the composition of the student population in such a way that weaker sections of society can get their fair share of opportunities

Views expressed by the Prime Minister on a subject are generally looked upon as being indicative of the official line of thinking, if not as statements of official policy So if Shrimati Gandhi's views are looked at in that light, we can look forward only to more of what has been going on for so long—more debate, more committees, more reports and substantive action, if any, so snail-paced that the passage of time will more than nullify its effects

The Prime Minister's warning on 'dislocations' in the existing process of education was unfortunate and really unnecessary For what use was it asking those who hardly move at all to go slow? And again, her linking of the present 'system' to the fact that India produces "exceptionally gifted" individuals was amusing because many Indians are able to achieve what they do in spite of the 'system' and not because of it It may be more relevant and instructive to investigate the number of talented Indians this system helps send into oblivion or nip in the bud each year   □

VERY recently, the first cigarettes at least partly made of substances other than tobacco were launched onto the market—in Germany and Switzerland One brand contains an American substitute, the other a German-made one Imperial Tobacco, which has 65% of the cigarette market in Britain and the largest volume turnover of cigarettes of any company in the EEC, now "hopes that its cigarette, containing its New Smoking Material (NSM) will be on sale to the public within 12 months Imperial (in conjunction with ICI) has spent £6 million on developing this tobacco substitute and a £13-million factory to produce 30 million pounds of it a year is nearly ready at Ardeer, Scotland The project is held up by the deliberations of the Hunter Committee —a body set up in 1973 under Dr R B Hunter to advise the Secretary of State for Health on guidelines for assessing and testing the risk/benefit ratio of the tobacco substitute, it has yet to report, though it gave Imperial the go-ahead for consumer acceptability trials of its product last August

Presumably on the basis of these trials the expected "new-smoke" (to use an Orwellian term for an Orwellian concept) is not likely to contain more than 20% of NSM—the rest will be standard cigarette tobacco I have smoked a cigarette made entirely from NSM and quite see the point It is supremely non-addictive In fact it resembles nothing so much as lighting the wrong end of a filter-tipped cigarette—which is perhaps not too hard to understand since filters are made of cellulose and so is NSM with the difference that NSM is toasted to reduce the

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Nosmoke without fire

from Angela Croome

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water content and give it a nice dark tobacco colour The brands launched elsewhere in Europe are much the same —it seems there is no substitute for wood—and one of the German lines has the appealing name "Nosmoke"

But with 80% tobacco, "no-smoke" it isn't Nor, one hazards, would a cigarette without nicotine satisfy the complex needs that a cigarette smoker seeks Curiously the massive research that Imperial has put into improving the smoking (including medical) characteristics of their cigarettes does not at present include measuring the nicotine level Tar and nicotine delivery are lumped together, and come out at 28 30 micrograms per cigarette as against only 7 micrograms for a cigarette consisting solely of NSM But Imperial's philosophy is explicitly that it is the tar that counts, and refers to the classical mouse-skin painting tests The company claims that not only is there less tar from NSM but that it is a different tar It is something that toxicology tests on NSM are being run by the independent laboratory with the greatest experience in toxicological testing, the Huntingdon Research Centre, and that screening there includes possible teratogenic effects It would be even more comforting to know that the Hunter Committee was going to recommend measures and standards of carbon monoxide delivery, implicated in heart disease which smoking is considered to accelerate—and that the committee was going to publish its report which it has no obligation to do

We are assured that NSM or "No-smoke" or any other semi-substitute cigarette will not be cheaper—"the Government cannot afford it", nor of course can the cigarette companies So the new-smokes must be sold on the "better for you" argument which on the evidence so far is specious Also one understands that cellulose from wood pulp (the nearest to a specific description of the new smoking material one can get) is cheaper than tobacco There is no prize for guessing who is going to be better off if the cigarette-smoking public can be persuaded to switch

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IT'S very exceptional indeed in the Netherlands that an essentially scientific dispute ends with people dragging each other into the courts Yet this is what happened when two Groningen archaeologists, Drs H Tj Waterbolk and D Stapert, accused a highly esteemed amateur, Tjerk Vermaning, of showing and selling fake Stone Age artefacts

Vermaning, a 46-year-old mowing machine repairer who has been living on an archaeology grant for several years, replied that one does not readily imitate (for example) a few hundred stone axes, that his accusers are of no scientific standing anyway, that he wants some "real archaeologists" to look into the question, and that he will sue Stapert and Waterbolk for libel

After being under arrest for two days, Vermaning was released

The whole affair seems to be the dead-end of a continuing story of trouble-laden cooperation of amateur and scientist Some ten years ago Vermaning gained national renown and became a local hero by digging up the remains of a Neanderthal camp site with several hundred artefacts in it, among which were various types of fist axes and a number of splinters The Groningen archaeologists could even restore some of the original firestone cnolls, from which Neanderthal man

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Neanderthal axes to grind

from Arie de Kool, Rotterdam

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had chipped his tools

Vermaning was very proud of his finds and expected rewards—not just the few hundred pounds that were paid for the stones, but an honorary doctorate and a staff appointment at the institute The university did not grant this to the astonishingly little educated man (three winters of elementary school, in summertime he had to help his parents), but they thought his talents sufficiently important to give him financial support Altogether this amounted to some £20,000 over several years

Vermaning decided, however, that this was not the recognition he was entitled to and he he refused to co-operate further—after he had found a new site, again containing several hundred pieces

Suspicions began to to grow, said Dr Stapert, when on a site where Vermaning had found more than 400 pieces, the archaeologists were not able to uncover one single chip more Vermaning uses this as an argument that the professional people are just not as com-

petent as he, but Stapert began to think that the 400-odd pieces that Vermaning found were just those he might have buried a short while before

His suspicion was greatly enhanced when he found what he thought to be traces of machining on two of the artefacts, and he became certain when he discovered that the typical shiny surfaces, supposed to be caused by weathering, were what he described as an easily removable recent kind of lacquering

He called in the police, accusing Vermaning of fraud—not just scientific but also financial, since he had sold recent imitations as genuine, old artefacts Vermaning was arrested and scientists, police and provincial government called a press conference

But Vermaning asked the police what proof there was in the statements of people who had refused to recognise him for his discoveries, which amounted to many times what they themselves had been able to dig up At the same time he accused the scientists of libel and demanded impartial evidence

In the Netherlands people are wondering who did make the axes, if not Neanderthal man, and also whether it was wise to cause a public rumpus before the matter had been dealt with in court □

UNDETERRED by the Arab boycott, an American firm with a stake in local science-based industry this week announced that it will be increasing its activities in Israel The firm, Miles Laboratories of Elkhart, Indiana, plans to invest an additional £8 million in its Israeli subsidiaries, which produce everything from the company's famous Alka-Seltzer to a broad range of sophisticated c h e m i c a l s for research laboratories

Two of the Miles' ventures in Israel are closely linked with local institutions of higher learning Miles-Yeda, located next to the Weizmann Institute in Rehovot, makes research chemicals based on Institute know-how These include lectins, spingolipids, affinity chromotography material, peptides, O^{17} and O^{18} stable isotopic compounds and a considerable number of immunochemicals At Ames-Yissum in Jerusalem, where Hebrew University research is utilised, diagnostic kits for testing the thyroid level in blood are the main product Export sales of these science-based firms last year hit £1 2 million

Miles, a "non-Jewish" company, was not attracted to the country by sentiment but by the chance of making a profit The Israeli Government realises this and does its part to encourage science-based industries by giving them loans and grants, as well as by fostering the establishment of special science-based industrial parks near universities and research centres

The conditions offered are apparently so attractive that many enterprises which could hardly be called science-based try to push their way into these parks In order to ensure that the parks serve the purpose for which they were established, the Ministry of Commerce and Industry announced this week that only enterprises which have ties with nearby academic centres would be allowed into them Those firms already in the parks will only be allowed to expand if they carry on a substantial R & D programme of their own

As elsewhere, orders from the military have played a key role in the development of science-based industry, notably in the electronics field Some achievements in electronics have been publicised, others are still under wraps

Publicity often comes when the firms concerned seek to enter the export market This is probably the reason why Israel Aircraft Industries, which already has multimillion pound overseas sales of rockets and planes to its credit, last week unveiled the Autocommander, a supersonic automatic pilot

Fully automated, the Autocommander relieves pilots of mechanical flight routine and enables them to concentrate on target pinpointing and other tasks while flying at more than twice the speed of sound It incorporates a reliable and fast-working detection system, including dual circuits, which ensure smooth functioning even if part of the device should fail Now being produced for the Israel Air Force, the Autocommander may become a major export item

● The military situation has also given impetus to research on war-related medical problems, such as that conducted by Professor Arye Weinrab of the Hebrew University and Mr Ezra Loewinger of the Hadassah-Hebrew University Medical Centre on the detection and identification of metal

Letter from Israel
from Nechemia Meyers

splinters which enter deep into the eyeball as a result of accidents or battle injuries Exact information about such splinters is important for surgeons, who must decide whether a delicate and difficult operation on the vitreous body of the eye must be performed

If the metal in the eye is steel, it may be removed by an electromagnet Chips of many other metals, though a source of irritation, can be left in the eye as they do not cause biochemical damage Copper splinters, however, present a special problem Sometimes the fragment of copper becomes encapsulated in the vitreous body of the eye and is rendered at least temporarily harmless On other occasions, the fragment of copper dissolves, releasing copper ions which, if they reach a certain level, can cause metal poisoning and an eventual loss of sight The doctors therefore require some means of keeping a constant watch on the copper level

Weinrab and Loewinger developed a system to detect the presence of metal ions in the eye by means of X-ray fluorescence The radiation is measured by a solid state detector which translates the X-ray energy into electric pulses of varying intensity according to the metal present Before the Yom Kippur War they were doing experiments on rabbits, when the war broke out and soldiers with eye injuries began pouring into Israeli hospitals, the metal detecting test was tried out on them
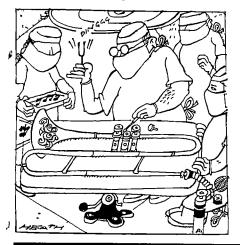
In many of the cases involving tank crew members, copper was found It came not from the tank itself (which is made of steel), but from the copper sheath near the head of the Russian anti-tank missiles hurled at the tanks The copper melted at the high temperature of detonation, so almost every steel splinter in a tankman's eye was either encased in copper or had copper adhering to it

● The severity of eye injuries during the war has led some of the country's leading ophthamological experts to recommend that all members of armoured and motorised units wear a special plastic shield Such a shield, already used by Israel Air Force pilots, is made of a plastic compound ten times more resistant than glass to high velocity particles It is also light, translucent and does not cloud with water vapour

● This shield will, of course, be of no benefit to men who have already lost their sight in combat, but attention is also being paid to their problems Chemist Avraham Schwartz of the Defence Ministry's Weapons Development Authority, using spinoff from research on plastic materials, has developed a process for printing books for the blind cheaply, quickly and in multiple copies In the new process, typewriters punch tapes similar to those of teleprinters, instead of pressing out the usual Braille reliefs These tapes are mounted on paper, and plastic is poured over them, penetrating the holes The raised dots that result can be printed on both sides of the sheet of paper, which itself serves as a relief plate for reproduction of the "printed" material

● The recently suspended talks between Israel and Egypt prompted several dozen scientists to recall an off-the-record discussion they had had some ten years ago with Henry Kissinger, then visiting Israel as a private citizen It was a discussion with special relevance to the question of how much trust can be placed in US guarantees of an eventual settlement, should one be reached

The conversation occurred at a time when Kissinger was campaigning against the proliferation of nuclear weapons, and so he naturally urged the scientists present to oppose the development of such weapons by Israel When Kissinger's presentation was completed, one of the Israelis put forward a question present in everyone's mind "If," the scientist said, "we were to accept your advice and forego the development of atomic arms in return for an American promise to assist us should we be under threat of attack by a nuclear power, could we be sure that such a promise would be kept?"

After pondering the query for a few moments, the Harvard scholar answered "No, you couldn't be sure American policy is made on an empirical, day-to-day basis It would depend on the circumstances of the situation when the crisis developed " One wonders what kind of an answer Kissinger would give today

Note paper follows?

A member of the physics department at the University of Surrey, Dr John Bowsher, must be working on one of the most unusual projects to be sponsored by the Science Research Council He is to bring the scientific method to bear on the burning problem of why really good trombones sound the way they do All trombones, it would seem, do not sound the same even though they may look indistinguishable, and it is generally the skill of a craftsman who knows just where to change the shape of a given instrument slightly that saves many new instruments from the ignominy of being $\beta+$ rather than α

With the music company Boosey and Hawkes, who produce 300 trombones a week, relying on the skill of just one craftsman in his 50s to suggest a little judicious adjustment in such and such a place, Dr Bowsher seems almost to be working against the clock in his analysis of the 'transient qualities' of trombone notes—namely what goes on in the first and last few milliseconds Fortunately Dr Bowsher and one of his research students are actually trombone players themselves □

correspondence

Czech conditions

SIR,—The discussion in *Nature* on the plight of the Czechoslovak scientists and my participation in it has had very unexpected and grave consequence for me personally

A few days ago I learnt of a decision of the Czech Ministry of Inner Affairs, according to which I have been stripped of my Czech citizenship The motivations for such an extraordinary decision are given as follows

(1) The lecture about human rights I gave at the annual meeting of the German section of Amnesty International in June 1974 in Duisburg (2) My letter to *Nature* (September 20, 1974), which, I have now learnt, had been broadcast in Czech by Rado Free Europe

Needless to say, I protest strongly against this decision, which I consider unlawful and contradicting the Universal Declaration of Human Rights as well as other generally valid and accepted declaration and principles

After being unemployed for more than three years because of my public activity and statements I was allowed to leave Czechoslovakia temporarily in December 1973 When leaving my country I submitted letters to the President of the Czechoslovak Academy of Sciences and to the Ministry of Education, informing them that I was leaving only because I could not find a proper job there, that I would be working abroad as a Czechoslovak scientist and citizen, and that I wished to return home as soon as a job was offered to me

The decision of the Prague authorities provides another strong argument against the opinion that things are being normalized in Czechoslovakia, it shows what is the real situation and the con-ditions Czechoslovak intellectuals have to live in

Yours faithfully,
FRANTISEK JANOUCH
Stockholm

Citation analysis

SIR,—Concluding their recent letter (March 13), Pragier and Ronayne, citing Langrish, state " our results raise serious doubts as to the validity of citation analysis in the determination of the relationship between science and technology "

I suggest that these conclusions have been reached because of a misconception about the scope of citation analysis, and do not, in fact, cast doubts on validity

Langrish examined citations from articles by British industrial chemists, finding that they were largely of non-university origin, whereas Pragier and Ronayne studied citations from biologically oriented articles in *Reports on the progress of applied chemistry*, finding them to be mainly of university origin

From this it may be concluded, as Pragier and Ronayne state, that information was drawn from different sources by these two communities, but this does not mean that such studies are not of value for studying the background of university and industrial science and technology

What it does mean is that such studies are likely to be most useful when applied to a community whose members are characterised by having an interest in a specific aspect of science

If the same pairs, triples, quads , of references are observed in a number of articles, there is an implied consensus of opinion—the authors are perceiving a relationship between the earlier cited articles The heavily co-cited articles form the putative 'core' literature of the subject This technique, originated by H Small, was used to study the literature generated by the scientific community engaged in amorphous (chalcogenide glass) semi-conductor research from 1968 to 1973 Until 1972 the core articles, with one exception, came from universities

In 1972 the core was augmented by articles from non-university sources The cause of this was heavy co-citing of 1972 articles by 1973 authors These authors often used title words indicating that device applications were described in their articles It may be concluded that during this period materials, hitherto of research interest, started to

A hundred years ago

Dingler's Polytech Journal contains an account of researches made by Dr Otto Krause, of Annaberg, on tobacco smoke, which he finds contains constantly a considerable quantity of carbonic oxide The after effects of smoking are said to be principally caused by this poisonous gas, as the smoker never can prevent a part of the smoke from descending to the lungs, and thus the poisoning is unavoidable The author is of opinion that the after effects are all the more energetic, the more inexperienced the smoker is, and he thus explains the unpleasant results of the first attempts at smoking, which are generally ascribed to nicotine alone

from *Nature*, **11**, 456, April 8, 1875

be used in devices having practical applications

Articles identified in this way may be depicted as interconnected elements on a citation map Replotting of the maps periodically enables the growth, decay and changing interrelationships of specialities to be studied It seems likely that this will provide an overview of the ramifications of science for the benefit of science policy makers and others

A E Cawkell
*Institute for Scientific Information,
Uxbridge, UK*

Great Plains weather

Sir,—A number of authors have pointed out the periodicity or quasi-periodicity of 20 to 22 years in the recurrence of droughts in the Great Plains of North America, the region between the Rocky Mountains and the Mississippi River Perhaps most notable of these droughts was the "Dust Bowl" era in the 1930s which retarded the Great Plains agriculture and economy for a full decade Most authors have also pointed out that the mid-years of the droughts coincide fairly well with every other sunspot minimum, specifically the minimum in which the polarity of the leading spots in the Sun's northern hemisphere is changing from north-seeking to south-seeking

Since we are now approaching another solar minimum of the type mentioned, the question of the reality of the sunspot drought relationship is of great interest The dates of the four most recent solar minima of this kind, and the central year of the four most widespread droughts in the Great Plains, were as follows

Sunspot minima	Mid-years of droughts
1889	1892
1912	1912
1933	1934
1954	1953

The causes of these droughts are not well understood J R Borchert has pointed out that some of these in the table were associated with greater than normal zonal circulation of the atmosphere, and some with increased meridional flow, evaluated on a hemispheric basis The common element in drought situations seems to be stability in the type of weather pattern, that is, drought tends to be associated with a highly persistent pattern, either meridional or zonal

I R Tannehill points out that drought in the Great Plains is associated with higher than normal pressure in the eastern Pacific area, whereas others find that droughts in western Kansas tend to occur simultaneously with positive height anomalies at 700 millibars over the eastern Pacific between latitudes 30° and 40° N and with negative surface temperature anomalies

over the eastern tropical Pacific

In the spring and summer of 1974 there was evidence for the beginning of another drought in the Great Plains If the situation is the same in most of the next several years, we may well conclude that the periodicity of 20 to 22 years is recurring A sunspot minimum of the type mentioned, is expected in, perhaps, 1976

In the spring the drought was confined to the western and southern parts of the Great Plains (Fig 1) Most of the Great Plains region was actually suffering from too much rainfall, with serious delays to the starting of the spring planting in large areas Meteorologically, the region was affected by greater than normal westerly wind flow Thus the drought in the area just east of the Rockies may perhaps best be described as primarily a rain-shadow effect

By mid-June and extending through July (Fig 2), the situation had changed to a more meridional flow, with a stable high pressure cell over the Mississippi Valley in the upper atmosphere This created an extended period of low precipitation and abnormally high surface temperatures just at the time when
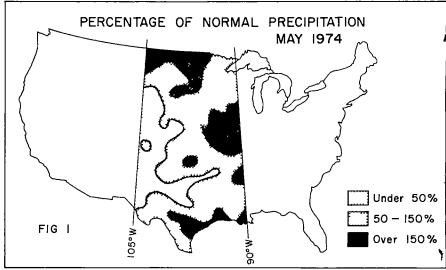
certain crops, such as corn and soy beans, were reaching the maturing stage For instance, Grand Island, in central Nebraska, reported no precipitation from June 15 until July 22, the longest rain-free period ever recorded at that station The resulting damage to crops in such states as Iowa, Kansas, Nebraska, Oklahoma and adjoining states approached $10,000 million dollars This loss was caused mainly to the extreme but short summer drought, but also in part by the spring drought in western Oklahoma and northern Texas
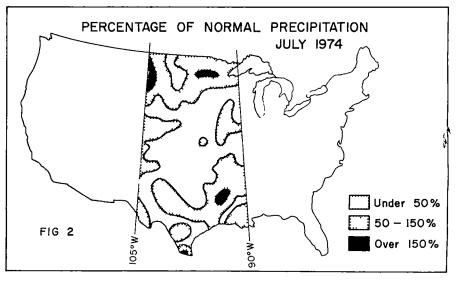
A mere coincidence in timing between the droughts and the double sunspot cycle will not, of course, constitute proof of a physical relationship If, however, the drought of the 1970s does materialise, over the next several years, we will have a very strong incentive to search for physical mechanisms to explain the linkage or to provide other reasons for the recurrence

Walter Orr Roberts
Roger H Olson
*University Corporation for
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Based on information from National Weather Service, NOAA

FIG 1

PERCENTAGE OF NORMAL PRECIPITATION
MAY 1974

Under 50%
50 – 150%
Over 150%

FIG 2

PERCENTAGE OF NORMAL PRECIPITATION
JULY 1974

Under 50%
50 – 150%
Over 150%

news and views

Wider implications of catastrophe theory

from Henry Chilver

THE interesting article you publish today by Thompson on 'experiments in catastrophe' (page 392) prompts me to make a plea for wider study, by both physical and social scientists, of a very important area of 'systems analysis' in which the concepts of catastrophe theory may well have an important part to play It will be evident to anyone who has attempted to 'design' a system—and particularly a complex system—whether this is in the field of engineering, organisation, planning, or in other fields, that modern design has encouraged designers to converge, in their ideas, on optimal systems

The forces at work to encourage designers to converge on optimal solutions are very strong All systems are based on ideas of increasing efficiency in one form or another for example, in his article Thompson demonstrates how the search for the lightest (and therefore most efficient) structural forms leads to potentially catastrophic engineering structures There are other examples in the fields of solids and fluids the breaking of bonds between solid particles leading to the catastrophic failure of materials, the breakdown of smooth flow around bodies, leading to turbulent flow

But there are many other types of systems—of widely different sorts—where catastrophe becomes more critical as design becomes more 'optimal' Many transport systems are highly sophisticated and optimised—and therefore highly sensitive to small perturbations which may lead to collapse or catastrophe One of the most sophisticated transport vehicles—the aeroplane—is itself a highly optimised system its structure is highly efficient and its power in flight is highly sensitive to the reliability of its engines, more optimisation comes into the picture during the operations of the aircraft, and the history of the aeroplane is very sensitive to any perturbations which lead to loss of control and thus to catastrophe, again, this particular mode of transport is highly sensitive to weather conditions, and fog at the airport may mean diversion to other airports, and, thus, to disruption of the system

But social systems themselves are 'planned' and 'designed' for equally critical states one of our present social problems is that we are optimising so strongly the complex interlocking and interdependence of institutions of many sorts that small perturbations of these systems can be catastrophic and lead to complete disruption States of employment (and unemployment) of vital minority groups are clearly very sensitive to small changes of economic parameters, and this suggests we have built highly critical systems of social institutions

Again, in manufacturing industry, the economy of production processes is highly sensitive to the volume of production, to the supply of crucial materials and components, and to the attitudes of small groups of staff For this reason, the present world decline in trade is giving some industrial manufacturing companies serious problems, because their continuing good health is so sensitive to the scale of production In such situations, sensitivity to small changes of the important parameters is wholly undesirable, but how can this be avoided if we are constantly forcing production processes to the extremes of low-cost and efficiency?

As pointed out by Thompson, Zeeman has shown some interesting catastrophe phenomena in natural systems Alternative animal states may be compared with the bifurcating paths of classical stability theory, although in such cases it may be very difficult to give numerate values to the many 'axes' or 'vectors' involved The state of good health of an individual is obviously an optimised condition—the 'design' in this case being reached through evolution and natural selection In the course of evolution, many inadequate optimal states have been weeded out, a possible reason for their being weeded out is that they showed high sensitivity to small perturbations of their systems In spite of this evolutionary process, the state of good health, in some respects, is still highly sensitive to a limited number of parameters, as, for example, to blood temperature and the working of a limited number of vital organs

Some aspects of ecology may well present problems of potential catastrophe The ecological balance in some systems is a delicate one perhaps between tropical forest and desert, or between alternative forms of animal life

In extending the concepts of Thom, Zeeman and Thompson to a wider range of systems one is tempted to look for a common thread between all these problems Following classical stability theory, we may search for 'potential functions' for all such problems, but this is not possible, since many of the common problems do not possess a simple potential function, nor, indeed, can be expected to possess them Another approach would be to construct simple models of these systems and apply general concepts of perturbation in studying their behaviour, this would bring catastrophe theory very close to general control theory

But one feature common to all these problems, and very correctly Thompson emphasises this, is that they are generally optimal systems Many are indeed highly optimal If we try to formulate a general problem it is something on the following lines as we pursue increasing optimisation in the design and planning of systems, how can we determine in simple terms those parameters to which the stability of the whole system is most sensitive? We might then follow this question with another is the degree of instability, or sensitivity to the critical parameters, an acceptable one?

Answers to these questions would be invaluable to designers of systems in many areas The teaching of such concepts as part of the philosophy of design would add a new dimension to our design methodology The ultimate goal in such studies is to determine common patterns between the many different systems we design and build, and in which catastrophe can occur What can be envisaged long-term is not only concepts of how to build optimal systems—indeed our knowledge of this is already very extensive—but how to study their sensitivity to disturbances and perturbations, and thus be able—as designers—to distinguish between highly critical and less critical systems

At the end of the day, of course, catastrophe cannot be avoided, but at least we should have a feel for the sensitivity of any optimal system to catastrophe Thompson's paper is a helpful pointer in this direction how can we encourage others in the same general direction?

Strontium isotopes and crustal evolution

from J Sutton

ONE of the most intriguing problems in geology at the present time is the investigation of the first 2,000 million years of the Earth's history We have now a detailed knowledge of small areas of crust dating from the latter part of the first 1,000 million years of geological time But the remnants of crust formed more than 3,500 million years ago are no more than a few tens of kilometres across at the most and are widely separated from one another

What we badly need is information on large scale activities within the primitive Earth, for this might link our fragmentary scraps of knowledge obtained from the ancient rocks exposed in Minnesota, west Greenland, southern Africa and Antarctica

Moorbath's paper in this issue of *Nature* (page 395) provides just such information, for the strontium isotope studies he reports here and those which Moorbath, Powell and Taylor have reported elsewhere (*J geol Soc Lond*, **131**, 213, 1975) bear directly on the question of the movement of material between crust and mantle in Precambrian times Moorbath reports very low $^{87}Sr/^{86}Sr$ ratios in the crustal rocks he and his colleagues have investigated and deduces that the rocks in question had formed from material which had been transferred to the crust from the mantle shortly before the times when they last crystallised He is in effect employing ^{87}Sr as a natural radioactive tracer to follow the circulation of silicates in Precambrian times This is now a well established technique but Moorbath's application of the method to the very old (roughly 3,700–3,600 Myr) rocks of Greenland and Rhodesia has produced results of

great importance for our understanding of the early Precambrian

The use of strontium isotopes in this way is in itself a fascinating concept Since the time the Solar System formed, ^{87}Sr has increased in abundance as ^{87}Rb breaks down, the rate of increase in any rock being governed by the amount of rubidium present The distribution of strontium isotopes through the Earth is therefore influenced by the manner in which rubidium and strontium are associated in rocks Since rubidium can be admitted to K^+ sites in the main rock-forming potassium-bearing minerals it is widely distributed as a trace element in the potassium-rich granitic rocks abundant in the upper crust but is much less abundant in the potassium-poor basic and ultrabasic rocks of the lower crust and mantle Study of the ratio $^{87}Sr/^{86}Sr$ can provide critical information as to the origin of an igneous rock A melt derived from the mantle will be marked by relatively low initial ratios of $^{87}Sr/^{86}Sr$ (0 699 from 4,600 million years ago to about 0 704 at the present day) In contrast in continental crust rubidium-rich granite can accumulate ^{87}Sr much more rapidly as the figures illustrating Moorbath's article indicate Accordingly a melt derived from granites which have resided for long periods of geological time in the crust may inherit this high ratio as an initial feature and so be distinguished from rock derived directly from the mantle Moorbath has been able to show that the initial $^{87}Sr/^{86}Sr$ ratios of the 3,700–3,600 million years old Greenland and Rhodesian rocks are so low as to suggest derivation directly from the mantle of those times He has also

found, as have earlier investigators of rocks formed rather later in the Precambrian, that rocks developed between 2,800 and 2,500 million years ago are marked by low $^{87}Sr/^{86}Sr$ ratios These are close to, or at most a little above, values expected of the mantle at that stage in the Earth's history

Moorbath concludes that these younger Precambrian rocks, some of which come from Scotland and Greenland, could not have been derived, as has been suggested, by the reworking of very much older granitic crustal rock such as the 3,700 million year old examples of west Greenland Moorbath follows Hurley in concluding that these facts indicate a progressive addition of mantle material to the crust He points out in the concluding paragraphs of his article that such transfers of material from mantle to crust seem to have been especially active at certain periods of time during geological history Some of these periods, it may be noted, coincide with the abrupt changes in continental movement indicated by the hairpins in the apparent polar wander paths established by palaeomagnetic investigations

One question raised by Moorbath's work with its additional evidence for a near identity of crustal and probable mantle $^{87}Sr/^{86}Sr$ ratios through early Precambrian time is of particular interest Do these results indicate, as Armstrong has proposed, that matter was exchanged more effectively between mantle and crust during the first 2,000 million years? Perhaps at that time no extensive portions of the crust remained isolated for sufficiently long to establish $^{87}Sr/^{86}Sr$ ratios significantly higher than those of the mantle

What came out of Kohoutek's comet?

from J F James

A YEAR and a quarter have now passed since Kohoutek's comet passed perihelion with awful anticlimax and if we have learned nothing else, we are now warned once again of the danger of letting empiricism masquerade as science With the nine months' warning of its approach there was ample time for elaborate international co-operation to be arranged and in spite of the disappointment of the amateurs and the disillusionment of the public the professional astronomers received it with a great broadside of astronomical equipment and studied it more comprehensively than any previous comet

Many of the more important obser-

vations have been reported in the December 1974 issue of *Icarus* (**23**, No 4), which was especially dedicated to the comet, and the editors have promised more later on

The discovery was made by Dr Lubos Kohoutek on March 21, 1973 and from three positions, one from a pre-discovery plate, Dr Brian Marsden published a preliminary orbit on March 26 (IAU Bulletin no 2514) From its position and magnitude at the time of discovery and from an empirical formula used to predict the future brightness of comets, it was concluded by some astronomers that it would be very bright indeed at perihelion and would become even brighter later on

as the tail developed In fact it did get to magnitude −2 3 at perihelion and was observed at the time by the crew of Skylab IV, 200 miles above ground It was of course invisible at ground level because of the glare of sunlight

Curiously there was no elaborate series of observations from Skylab IV and the observers there used the archaic method of making drawings of the appearance of the comet These drawings were sent down to Earth by television link Skylab's crew were the first to observe the tail of the comet and also found the 'anti-tail', a sunward-pointing spike like that of the Arend–Roland comet of 1956 As the

comet drew away from the sun after perihelion passage this spike became less intense, and was only very faintly visible to photographers in mid-January, the time when the comet was most favourably placed for ground-based observations By then the spike was much attenuated and more like a fan The spike is thought to be part of the dust-tail of the comet, comprising particles travelling in a different direction from, but in the same line of sight as the head The dynamics of these spikes are well understood following the theoretical analysis of Finson and Probstein some years ago, in *The Astrophysical Journal*

More remarkably, the Skylab observers noted that the tail near perihelion was bright yellow It is hard to see how this could be anything other than resonance scattering of sunlight by sodium vapour and the fact that it was bright enough to be seen by the colour-sensitive cells in the retina bears witness to the comet's magnitude at perihelion

The comet failed to develop a marked dust tail after perihelion passage and this was the cause of all the disappointment Observers below 40° latitude who knew where to look and what to look for could just see the comet with the naked eye It was an easy object in 7×50 night glasses, but never spectacular This did not stop a NASA team (Hyder, Brandt and Roosen) from taking some very fine detailed photographs showing structure in the tail and showing the separation of the dust and gas components

The chief investigation seems to have been, by common consent, the search for molecules and the analysis of dust in the tail The central problem—the nature of the head—was not attacked directly, chiefly because the comet was never close enough for any object smaller than about 500 Km across to be resolved In any case, this is not a problem likely to be solved by anything less than a comet interception probe—a spacecraft designed to 'fly by' at a close distance from the head or even to collide with whatever is at the centre of the head (This is something that will be well worth doing when a comet comes close enough and enough warning is given to make it feasible) Thus the contest between the two theories of the nature of the head, the 'dusty snowball' and the 'dense cloud of dust' is no nearer a decision, but the dusty snowball model, the current favourite, received a knock when Traub and Carleton of the Smithsonian Institution searched for and failed to find either water vapour or methane, the chief materials likely to form the 'snow' of the snowball

Various people found and measured the familiar cometary molecules, such as C_2, CHOH and CN and new tech-

niques were used for some of these observations For example, Blamont and Festou of CNRS observed OH by resonance scattering, in the infrared, of the lowest electronic transition band of this molecule and HCN was detected by its radio emission in the 3 mm band by a group from Los Alamos, Boulder and Green Bank More complicated molecules were also observed For instance, Ulrich and Conklin reported radio emission from methyl cyanide (*Nature,* **248,** 121), and other groups made searches at radio wavelengths Atoms too were identified by their spectra, sodium and hydrogen being measured by a group from Bell Laboratories and Earlham College, who simultaneously searched for and did not find any trace of helium Hydrogen emission at Lyman-α (1215 6 Å) was used to photograph the head, both from Skylab and from a rocket-borne camera This is the brightest of all resonance lines, and showed an enormous halo of about 4° diameter, strongly condensed towards the head of the comet It was possible to measure the outward velocity of this hydrogen by searching for the moment when its velocity relative to Earth was zero and the light, not shifted by Doppler effect for the moment, was absorbed by the hydrogen in Earth's geocorona This outward velocity was lower than expected, sufficiently to indicate that the hydrogen was the product of OH dissociation rather than H_2O dissociation

The dust emitted by the comet received its share of attention but produced nothing startlingly new E P Ney of the University of Minnesota saw once again the 'silicate bump' in the infrared emission spectrum of the dust In the 8–13 μm 'window' in the Earth's atmosphere the radiation received from the dust is predominantly its own thermal emission rather than scattered sunlight The black-body curve is followed except near 10 μm where there is an excess of intensity This has been found in the laboratory to be characteristic of metallic silicates, and has been observed in interstellar dust grains as well as in comet tails Curiously the bump is missing in the spectrum of the anti-tail of Kohoutek's comet

To sum up although the public was disappointed the astronomers were not, and if the comet was unremarkable, this was in great contrast to the number of novel and powerful techniques that were used to study it As in all good investigations, more questions have been raised than answered, and what we all pray for now is a modest comet passing close to earth with plenty of advance warning Then we might really know what comets are made of and what comes out of them

Trying to smooth background radiation

from A C Fabian

THE two main handles that we have on background radiations are provided by measurements of spectrum and isotropy Data on these have been well marshalled by students of the microwave background to show that the Universe has passed through some hot, early phase The other major background radiation occurs at X-ray frequencies, and its origin is still unknown, although it is reasonable to suppose that most of it is extragalactic and originates at cosmological distances One problem in interpreting observations of small angular scale (about 5°) fluctuations in the X-ray background stems from the point that these fluctuations arise from those sources just not resolved A glance at the *Uhuru* catalogue of X-ray sources and recent literature shows that a growing number (~30) of extragalactic objects such as clusters of galaxies, radio galaxies and QSOs are detectable by their X-ray emission This means that the more distant and weaker members will contribute to the fluctuations, and indeed most of the fluctuations will be due to those sources having intensities such that there is about one per detector beam in the sky They need not necessarily be at cosmological distances, and so the fluctuations may not directly assist in the quest for the source of the X-ray background

It has been suggested that the fluctuations observed with *Uhuru* are less than would be expected from the counts of sources already detected This can then be used to suggest that some of the as yet unidentified X-ray sources are really at cosmological distances and that perhaps we are seeing to the edge of the Universe Other possible interpretations include consideration of the distribution of the sources in the sky, for they may form some crude lattice or at least anti-clump This means that the formation of one source may inhibit the formation of any other sources nearby

D A Schwartz of the Center for Astrophysics, Cambridge, Massachusetts has posed the question "Can the constraint of finite mass smooth fluctuations in the background radiation?" (*Astrophysical Journal,* **194,** L139, 1974) The idea here is that the Universe may be considered composed of volume elements each containing an exactly equal finite amount of mass This mass is then divided between all known objects including X-ray sources At the extreme, if all the mass goes into X-ray sources the sky must take on a roughly lattice structure and even

if non-X-ray matter is allowed some constraint is still placed upon the X-ray source distribution Schwartz notes an analogy with the Fano effect, which applies to the number of ion pairs produced by a fast particle in a detector chamber The distribution of the number of ion pairs is tighter than the Poisson distribution often applied to such problems He has taken the formulae for this effect over to his work and attempts to show that smoothing is possible and believes that it is not unreasonable that a factor of two smoothing should emerge No assumptions are made about the distances to sources and it appears that the principle is to be taken quite generally

What Schwartz has not done is demonstrate that his approach is consistent and that he is justified in using a method from a different branch of physics Consider a Universe consisting of constant mass elements of some fixed scale size, within which any dispersion of the number of X-ray sources is restricted in some way Each volume element contains one or more sources but an X-ray detector will observe fluctuations which will be dominated by the distance from the observer where there is about one source per beam The beam will therefore only observe a small portion of a volume element at one given time, so it matters whereabouts in that element the sources are situated If they are all in one corner, very large fluctuations will result! The Fano effect does not translate directly to this problem, since the positions of the ion pairs in a detector chamber is irrelevant

We can conclude therefore that the finite mass constraint does not smooth background radiations to the extent suggested by Schwartz If the real situation is compared with the artificial one just discussed, it is hard to see that any significant reduction will result at all

Predators' good housekeeping

from our Animal Ecology Correspondent

PREDATORS, in their concern to maintain a supply of food, rarely eat more than a small proportion of the population of their principal prey Kruuk and Turner (*Mammalia, 31*, 1, 1967), for example, calculated that lions consumed annually no more than 3% of the wildebeest stocks—their main prey —in the Serengeti If this did not supply enough food, alternative sources were tapped Similarly Pearson was able to show that feral domestic cats exerted a powerful pressure on declining rodent populations but switched to alternative

prey when these became too scarce (*J Anim Ecol*, 35, 217, 1966)

For large predators, such as lions and cats, a switch to alternative prey may be far easier than for small predators which are specifically adapted for feeding on a restricted size range of prey Brown and Lasiewski have drawn attention to the problems facing weasels in northern tundra regions (*Ecology*, 53, 939, 1972) Males and females, being of markedly different sizes, exploit different feeding niches and thus function much like two separate species of small predators Perhaps this size dimorphism makes existence possible in these conditions, or at least permits higher populations Iverson has pointed out that weasels have a metabolic rate between two and three times higher than that of other mustelids, and a food requirement to match (*J cell comp Physiol*, 81, 341, 1972) The ecological implications of an inability to switch to alternative prey coupled with an unusually high food demand are twofold First, weasels might be expected to be highly territorial with each territory containing sufficient amounts of the correct prey species, and second, they might be expected rapidly to decrease breeding when food becomes too scarce in order to conserve stocks

Erlinge of Lund has recently published some interesting data on territoriality and population size of the weasel in relation to prey abundance (*Oikos*, 25, 308, 1974) His study area was 80 ha of spruce plantation, both young and old, deciduous woodland and replanted clearings Various stone walls and dense cover throughout rendered the whole area a most favourable weasel habitat Weasels were trapped and marked by ear-clipping during two autumns and one summer The very high number of recaptures revealed that in the autumn of 1972 the habitat was divided up between three resident territorial males, two females showing evidence of having suckled young, and six young weasels Three other males visited the area as transients During the winter of 1972–3 the territorial system was much as it had been the autumn before but a rapid turnover in territory ownership was evidenced by only one of the autumn residents being a territory holder the following summer

In the autumn of 1972, most weasels had been caught in the spruce plantation The population of short-tailed voles was then between 30 and 40 per ha By the following summer the vole density dropped to less than 10 per ha with the result that most of the residents left the plantation and settled in the replanted clearing where the rodent population was some 60% higher The emigration of all the females from the

study area later in the summer of 1973 meant that there was no breeding

East and Lockie have made some observations on the food requirements of captive breeding weasels (*Proc zool Soc Lond*, 143, 395, 1964) A pregnant female weasel needs 20–30 g of mouse each day for about 35 days, and during the 21 days of lactation she requires about 60 g each day Once the litter of, say, six young weasels starts eating meat the requirement of food increases over the next 40 days from 69 g to 192 g daily Translation of these figures into rodent densities requires the acceptance of certain assumptions, namely, that each vole weighs 25 g, that breeding female voles produce 0 125 young each day per adult in the population, that a generation time is 45 days and that the sex ratio is equal These assumptions accord well with the many published observations on vole productivity Given them, more than ten reproducing voles are necessary to produce sufficient food for one breeding weasel

It seems that when the prey density in Erlinge's study sank to less than 10 per ha, breeding was inhibited by the emigration of lone females Given that the average territory size of females is about 1 5 ha and of males considerably larger, it is hard to see that the reported rodent densities could not support both sexes especially if there is any differentiation in feeding niches One must conclude that such differentiation is perhaps less clearly defined in rich woodland areas than in the more rigorous northern latitudes Erlinge's data highlight some of the problems faced by highly specialised, small predators, and some of the difficulties encountered by ecologists in interpreting them

Noisy meteors

from David W Hughes

THE sounds associated with bright meteors and fireballs can be divided into two fundamental types—a single or repeated sharp cracking noise and a distinct rumbling, these occurring a few minutes after the passage of the meteoroid—and a second much rarer noise, a hissing sound reported to be heard almost simultaneously with the visual phenomenon
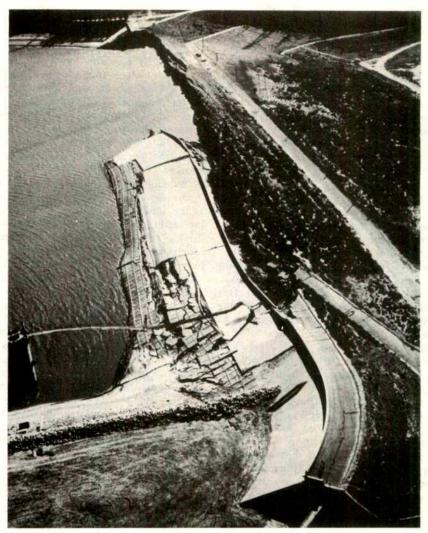
Theories about the origin, and problems associated with measuring the first type of meteor noise are discussed by ReVelle in the February edition of *Sky and Telescope* (49, 87, 1975) He concludes that only fireballs brighter than visual magnitude −8, with kinetic energies of the order of 10^{15} erg can produce sounds These penetrate to the lower levels of the atmosphere where

the air is dense enough to act as a fluid and therefore to sustain organised sound waves. One example quoted is the large fireball seen and heard by Wilson at College, Alaska. The meteor at a range of 300 km produced an airwave signal of maximum amplitude 4.6 dyne cm^{-2} which lasted for 12 s. The radius of the strong shock region surrounding the train was estimated to be about 450 m, the incident meteoroid having an energy of 6×10^{20} erg. Assuming a density of 0.3 g cm^{-3} and a geocentric velocity of 11.2 km s^{-1} the meteoroid had a mass of 9×10^{8} g and a diameter of 13 m.

During atmospheric entry the meteoroid can be travelling at 40 to 250 times the speed of sound. The hypersonic flow of air around the meteoroid produces an effect analogous to the explosion wave produced by a cylindrical line source. As the disturbance propagates outward from the meteor it eventually becomes weak enough to be described as a sound wave. The frequency is, however, usually below the audible limit of the human ear and all that can be detected of this infrasonic wave is a sharp 'crack' as it passes due to the rapid pressure change.

The transmission of the sound wave to ground level depends on the temperature, density and wind profiles of the atmosphere. In general the steeper the angle of entry of the meteoroid the less likely it is for a ground observer to hear the sound. Winds in the 40–60 km region can produce channelling effects enabling the sound to propagate over unusually long distances. Atmospheric damping of the wave energy increases with frequency, which accounts for the low frequency rumbling sound analogous to thunder, heard after the passage of the meteoroid. Meteor sounds have characteristic frequencies from about 0.05 to 5 Hz. In general the more energetic the meteoroid the lower the frequency of the sound wave produced.

ReVelle describes how microbarographs are to be used to detect and measure infrasonic sounds from meteors. These are designed to detect pressure amplitude changes in the range 0.1 to 100 dyne cm^{-2} with frequencies between 10^{-3} and 10 Hz and will be installed in the areas of the North America prairies covered by the meteor cameras of the Smithsonian Astrophysical Observatory and the National Research Council of Canada. From known incident fireball fluxes ReVelle expects to record about 10 meteor sounds per microbarograph station during 6 months of night-time observing. Wind noise is one of the major problems but filtering techniques can reduce this to a tolerable level provided the wind speed is less than

THE upper and lower Van Norman dams, which lie in the northern San Fernando Valley about 32 km north-west of Los Angeles, were built between 1912 and 1921 with hydraulic fill placed on young valley alluvium. During the 1971 San Fernando earthquake (magnitude 6.5) both were badly damaged by slope failures on the reservoir embankments. This US Geological Survey picture shows the lower dam just after the earthquake. The 1971 shock was accompanied by the most intense ground motions ever recorded instrumentally for a natural earthquake. But on the basis of geological and historical studies, R. F. Yerkes of the US Geological Survey estimates that earthquakes with magnitudes of at least 7.7 may be expected in this highly populated area. A magnitude 8+ event on the San Andreas fault about 40 km away could also cause extensive damage in the Van Norman area.

10 m.p.h. The recordings will yield energy-against-frequency characteristics of the sound waves and should provide new clues about the meteoroid ablation processes in the lower atmosphere and the complicated interactions which take place at the meteor train–atmosphere boundary.

The second type of noise, the hissing, swishing, buzzing sound heard at the same time or just before the fireball is seen is much more difficult to explain. Such noises have been termed 'anomalous' because they seem to be transmitted at the speed of light. Romig and Lamar in a detailed study of the phenomenon (*Rand Corporation Memorandum* RM-3724-ARPA) have

ruled out the possibilities that the noises are just imagined by the observer (unconsciously associating the fireball with a skyrocket for example) or just chance coincidences. Many trained observers have heard these anomalous sounds and the report contains many examples of people who heard a whizzing noise, looked up and then saw the fireball.

How can this sound be produced? Two other natural phenomena might provide clues. Lightning leader strokes produce brontophonic sounds heard just before the thunderclap from the return stroke. Also aurorae have been reported to produce hissing sounds. Brontophonic noises are produced by

small coronal discharges from surrounding vegetation but it seems very unlikely that enough free charges could be deposited by the fireball passage to explain the noise. The origin of auroral noise is still a mystery.

Considerable radiation must be produced by the fireball in regions of the electromagnetic spectrum other than the visual and this could provide a possible explanation. Electrophonic sounds for example have been heard by people exposed to beams of low powered radar sets. These have been described as buzzing, clicking, hissing or knocking sounds and seem to depend on the transmitter characteristics. An electromagnetic power density as low as 400 μW cm^{-2} can be heard, but only by people whose audible hearing by air or bone conduction was good above 5,000 Hz. Perhaps radio frequency electromagnetic waves are acting directly on the brain or inner ear. There are distinct similarities between electrophonic noises and anomalous sounds from fireballs but the production mechanisms of both are still unknown.

With luck the bariophonic recorders will pick up examples of anomalous noise as well as the more normal meteor sounds and help solve this problem also. One snag is that anomalous noise seems to be associated with brighter fireballs (average magnitude −13) which are rare objects indeed.

Plate tectonics and general relativity

from Peter J. Smith

THE mechanism responsible for the movement of lithospheric plates over the Earth's surface is a subject of continuing debate. The majority view is that plate tectonic processes are related to convection in the mantle, although there is less agreement about whether convection is the prime mover or a secondary consequence. If the prime mover, convection would be directly responsible for the motion of the overlying plates, and that of ocean floors in particular; but it is also possible to envisage that plates drift for other reasons (for example, by sliding under gravity) and that 'convection' arises from the need to complete the flow patterns at depth. The latter hypothesis bears some resemblance to the minority view of Van Bemmelen (*Tectonophysics*, **1**, 385; 1965) and others, whose 'undation theory' proposes that selective high radioactive heating in the mantle produces warping of the overlying crust followed by lateral spreading under gravity.

But whatever the disagreements over

causes, there is general consent that plate tectonic processes are long term phenomena with characteristic time scales of at least hundreds of millions of years. By contrast, the high seismic activity which defines the locations of plate boundaries is sometimes claimed to have much shorter periodicity of about 11 years (see, for example, Simpson, *Earth Planet. Sci. Lett.*, **3**, 417; 1967). Machado (*Geol. Rund.*, **64**, 74; 1975) has now drawn attention to another example of this supposed periodicity—in the seismicity of the Azores—which he finds 'informative'. What makes the Azores particularly interesting is that they lie at the triple junction of the American, African and Eurasian plates and are thus presumably influenced both by the constructive margin forming the mid-Atlantic ridge and by the destructive margin along the Eurasian–African boundary. Machado finds that the 11-year variation in earthquake frequency is apparently present in data both from the islands of Fayal and Pico and from the islands of Terceira and San Miguel. But there is a difference in that the main seismic swarms on the two pairs of islands alternate, the main Fayal–Pico swarms being associated with crustal expansion (constructive) and the main Terceira–San Miguel swarms being associated with contraction (destructive).

Machado generalises from this example by suggesting that expansion and contraction perhaps alternate within an 11-year cycle everywhere. He then goes on to propose that this alternation could be due to 11-year pulsations in the gravitational constant (G). Indeed, he does more than propose; he claims to show mathematically that gravitational pulsations are a necessary consequence of general relativity within an expanding Universe. The physical effect of such pulsations would be alternate expansions and contractions not only of the Earth's lithosphere but of the whole interior. During an expansion phase, rifting would take place at oceanic ridges and mantle material would rise to fill the fractures; during the subsequent contraction phase, excess lithosphere would be forced downward at subduction zones. The net effect would be a drift of the plate; but as envisaged by Machado, the mechanism avoids the paradox implicit in attempting to explain apparently simultaneous expansion and contraction by a variation in G. In fact, there is no simultaneous expansion and contraction; each process takes place over alternate 5.5-year half cycles.

It is difficult to know how far to pursue an idea such as this. Is there really an 11-year periodicity in worldwide seismicity? And if so, do worldwide constructive and destructive processes alternate over half-cycles

within each cycle? On the other hand, it would perhaps be unwise to reject the idea of short-period gravitational fluctuations out of hand. The idea of a long term decrease in G has a long and distinguished history; and there is still a lingering suspicion in some quarters that gravitational variations may ultimately be found to account for the Earth's mobility as manifested at the surface by activity at the three types of plate boundary.

Folding proteins along the dotted lines

from Barry Robson

THE relation between the amino acid sequence and conformation of a globular protein is as yet imperfectly understood. But the fact that many proteins can spontaneously fold into their biologically active conformations suggests that computer simulation of the folding process would be likely to throw considerable light on that relation. An interesting technique for carrying out such a simulation has been proposed by Levitt and Warshel in *Nature* (**253**, 694; 1975).

The work of Levitt and Warshel may be considered as an extension of the study by Ptitsyn and Rashin (Preprint, Acad. Sci, USSR., 1973; *Dokl. Acad. Nauk SSSR*, **213**, 473; 1973). Certainly both investigations neglect the polypeptide backbone of the protein, and treat the amino acid side chains essentially as simple single-centred structures with interaction energies calculated from amino acid solubilities in ethanol. In both cases, therefore, emphasis is placed on the hydrophobic interaction between side chains as the principal driving force towards a biologically active conformation. The more recent study, however, is considerably more ambitious. Ptitsyn and Rashin confined their attention solely to the amino acid residues within the extensive α-helical regions of myoglobin, and fixed the conformation of these residues as α-helical from the outset. Levitt and Warshel, on the other hand, propose a technique in which the backbone is free to adopt any conformation.

The problem of manipulating the backbone without having to represent it in detail is overcome by the ingenious use of a device due to the Flory school (P. J. Flory, in *Statistical Mechanics of Chain Molecules*, 248–306, Wiley, New York, 1969). Flory replaced the actual molecular bonds of the backbone by virtual bonds connecting the α-carbon atoms at the base of each side chain. Each virtual bond, represented in the publications of the Flory school as a dotted line, substitutes for one fixed

and two rotatable molecular bonds Levitt and Warshel propose that the simplified representation of a protein as a set of side chains connected by virtual bonds between their α-carbon atoms is an adequate model for simulating the folding process In other words, they propose that the computer should literally fold up a protein along the dotted lines

The authors test this representation by simulating the folding of pancreatic trypsin inhibitor, a small protein of 58 amino acid residues and hence 57 virtual bonds Since the most stable conformations of a molecule are those of least energy, a procedure is used which minimises the conformational energy as a function of the angles of rotation around the virtual bonds Unfortunately, the procedure is one which does not allow the folding pathway to cross low energy barriers in the conformational energy surface, barriers which the protein in nature would be expected to negotiate readily To avoid trapping the molecule in a conformational energy well which is surrounded by low barriers, a technique called 'normal mode thermalisation' is used after each minimisation to generate a new random starting point close to the well The authors do not, however,

allow for the possibility that during minimisation the simulated pathway of folding could be confined to a trivially shallow channel, a difficulty which could be avoided by the use of a different kind of minimisation procedure The conformational energy surface of a protein is so complex that the use of certain traditional minimisation procedures may at worst resemble an attempt to plot the potential course of a stream by chasing a free-wheeling locomotive down a railway track Nevertheless, the authors make the exciting point that in some of their simulations, the protein arrives at a conformation close to that of the known, biologically active structure

One of the principal difficulties in this study is that the proposed model for a globular protein is such a drastic simplification As a further check on the validity of their model, the authors confirm that it is apparently stable when set in the biologically active conformation This is not, however, the sole criterion of an adequate model since the energy surface elsewhere may depart considerably from reality Another problem is that only very few different starting conformations for the folding simulations were explored, despite the fact that folding even from

the same initial conformation did not necessarily yield the same final conformation For these reasons there still remains the danger that the approximations to the biologically active conformation obtained by simulated folding are fortuitous, and an artefact of the model and starting conformations chosen

By far the most serious difficulty, however, is that the use of virtual bonds cannot by its very nature be a general solution to the folding problem Neglect of the backbone with its capacity for intramolecular hydrogen bonding means that α-helical regions, and possibly other secondary structure features, lose their intrinsic stability Possible exceptions are those helices with well defined clusters of hydrophobic or hydrophilic side chains on the helix surface, but such helices are by no means ubiquitous It is therefore notable that Levitt and Warshel, like Ptitsyn and Rashin, were obliged to start with the known α-helical region of trypsin inhibitor in the α-helical conformation and although the virtual bonds within the helical region were allowed to vary, the helix was the part of the real protein reproduced least well after folding As the authors point out, the various statistical pre-

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The psis and their relations

from David J Miller

—intermediate boson models, 'charm' models or 'colour' models

The charm or 'SU(4)' model (see *Nature*, **253**, 438, 1974, for a brief outline) is still the most popular Various schemes have been suggested to fit the ψ and ψ' into a multiplet representation of the group SU(4) In 1961 Gell-Mann and Nishijima succeeded in explaining many of the properties of hadrons by fitting them into multiplets of SU(3)—sometimes called the 'eight-fold way' because many of the multiplets contain eight particles If the new quantum number charm is allowed, then SU(3) can be extended into SU(4) in a very natural way—just as the previously well established SU(2) scheme of 'isotopic spin' became SU(3) when Gell-Mann added the 'strangeness' quantum number In a simple SU(4) scheme there is an obvious place for one ψ particle, in the same multiplet as the well established vector mesons ρ, ω and φ, though it is not so easy to fit in two If the ψ (3 1) is fitted in, then predictions have been made (by M K Gaillard, Rosner and others) that charmed particles called the F and the D should exist, with masses as low as

2 2 GeV/c^2 The psis could not decay into Fs or Ds, but the Fs and Ds could be produced in pairs with opposite values of the charm quantum number It has been suggested that the bump at 4 2 GeV/c^2 may be due to the onset of F or D production, but there is no direct evidence to prove this

A recent paper by a Toronto group (*Phys Rev Lett*, **34**, 541, 1975) suggests a novel way of fitting both the ψ (3 1) and the ψ' (3 7) into the same SU(4) multiplet as the vector mesons As well as charm, they invoke a new "medium strong" charm-breaking interaction They assume that the ψ' (3 7) is a combination of a charmed quark and a charmed anti-quark—the obvious assignment for a ψ in the vector meson multiplet Their new interaction allows them to make the ψ (3 1) from a different mixture of charmed and strange quarks and antiquarks As in other SU(4) theories, they predict other new charmed particles which should be seen soon, if they exist, if their scheme is correct the new particles should have extremely interesting properties due to the effects of the medium strong force Perhaps this force also has something to do with the medium strong properties of the ψ (3 1) in its decay and in photoproduction (see *Nature*, **254**, 180, 1975, for a discussion of these results)

diction rules so frequently applied to the prediction of helical regions could first be utilised in order to locate the α-helices Nevertheless, the predictions would have to be particularly good because without the capacity for helical hydrogen bonding, the backbone would presumably not be capable of realistically winding or unwinding to compensate for errors in the statistical predictions

These cautionary notes may well seem pessimistic in the light of future studies but the onus is on the authors to provide a more extensive body of evidence for the validity of their model, particularly evidence resulting from a much larger number of folding simulations If the technique can be justified it will undoubtedly prove of very great value for folding the non-helical regions of proteins and bringing preset helices together

The study of Levitt and Warshel reflects the ingenuity and optimism to be found in a number of recent publications relating directly or indirectly to the folding of globular proteins At meetings and in visits to other laboratories, one can readily detect the underlying tension and enthusiasm, there is an exciting smell of sulphur, carbon, nitrogen and oxygen in the air

Michael Levitt and Arieh Warshel reply— ROBSON sees our work as similar to that published by Ptitsyn and Rashin (*Dokl Akad Nauk SSSR*, **213**, 473, 1973) Ptitsyn and Rashin have made a physical model of myoglobin out of nine preformed helical segments connected by pieces of flexible chain They used regular plasticine cylinders for the helices (rather like the simplified models made by protein crystallographers from low resolution electron density maps), and manually searched possible types of packing after making some assumptions about the neighbouring helices along the chain coming together first The interaction energy of a pair of helices was obtained by counting which residues were taken out of water at the contact surface, hydrophobic residues preferring to be buried and hydrophilic residues to be exposed With this model they concluded that an arrangement of helices closely like native myoglobin had both the lowest energy and the most stable intermediates at each stage of the assembly We find Ptitsyn and Rashin's work exciting because of its originality and the unexpectedly clear-cut picture of stable subassemblies when forming myoglobin from helices It is surprising that their work is considered so similar to what we have done Our work is a complete *ab initio* computer simulation of the folding of a whole protein chain based on realistic chain geometry

and interatomic forces derived from studies of small molecules, and including both the dynamics of the chain and randomising effect of temperature In our paper (*Nature,* **253**, 694, 1975), we did not neglect the polypeptide backbone but only omitted the hydrogen bonds between peptides which may be weak in the early stages of folding when the protein has a fairly open conformation in an aqueous environment We said they could be introduced later, along with other detailed interactions It is certainly not true that we are 'obliged to start with the known α-helical region of trypsin inhibitor" (see page 697, column 2 of our article) We include the effect of the solvent using amino acid solubilities but only as part of a realistic energy function with contributions from van der Waals, electrostatic, and torsional forces

We did, of course, refer to Flory's work on the virtual bond approximation, which was designed and used for calculating statistical properties of disordered random coil polymers It was totally unexpected that this simplification could also be used in a complete simulation of protein folding It now seems that the protein chain can be simplified because the peptide group is planar and the two rotatable bonds almost co-linear Each value of our single variable torsion angle α replaces a pair of the usual backbone torsion angles φ and ψ, so that we can halve the number of variables and hardly affect the representation of the protein backbone It is unfair to say that we consider the protein as "side chains connected by virtual bonds" We connect adjacent Cᵅs by a rotatable virtual bond and the side chains then stick out from this backbone (see Fig 1) Omitting the backbone and connecting adjacent side chains by a virtual bond would be a very poor model indeed

We do not believe that the native conformation of a protein necessarily has the lowest free energy, but try to simulate the actual folding process For this one must move smoothly over the energy surface down the energy gradient The minimisation method we use, developed by the Numerical Analysis group at Harwell, has been proven to be very powerful for a wide class of problems, it follows the path down the gradient more efficiently than any other method We are well aware of the so-called "untraditional" non-gradient minimisation methods which could conceivably avoid shallow minima, but these would require between 50 to 100 times as many energy evaluations to reach a minimum What would be worse is that one would lose all physical reality and still have no guarantee of crossing low energy barriers Our normal mode thermalisation

technique uses information gathered at no extra cost while dropping into a minimum to get out again In fact very few shallow minima are encountered (usually about 1 or 2, see Fig 4) in folding from an open to compact conformation This is an expected result of averaging over less important detail, but may well represent, as we wrote, a real property of the first stage of the actual folding process The conformational energy surface of a protein seems rather simple to us, and the analogy of following a freewheeling locomotive is actually a close picture of what we find

Until now nobody has even shown that accepted interatomic forces give rise to a true minimum (net couples less than 10^{-6} kcal mol^{-1} rad^{-1}) near the native conformation of a protein Were we to have used the accepted all-atom representation of pancreatic trypsin inhibitor, our work would have cost more than NASA's Apollo programme, and the results would have been much harder to interpret because of the many shallow minima caused by the minor irregularities of the side chains Throughout this project we have been very aware of the necessity of making the calculations as efficient as possible, we have managed to reduce the calculation time by a factor of about 1,000,000 by (1) considering the most effective variables, (2) averaging over side-chain conformations, (3) using a very efficient minimisation method, and (4) using normal modes in the thermalisation

Since last August when we obtained the results published in *Nature,* we have extended the work considerably We have simulated pancreatic trypsin inhibitor folding including the backbone hydrogen bonds and get as good a fit to the native conformation We have also tested different starting conformations and sets of energy parameters, and still obtain a significant success rate The same methods have been applied to rubredoxin, another small protein, with encouraging results Tests on the "mainly helical" proteins myoglobin and myogen have shown that when peptide hydrogen bonds are included, helices are stable and capable of growth and that several helices can come together as in the native protein Now that we have a new version of the program two and a half times faster (one cycle for PTI in 0 2 s) we will look at bigger proteins, like lysozyme, ribonuclease, and the antibody domains

For the first time it is possible to simulate protein folding by computer study each step of the process in detail and test which forces dominate and which parts fold first A great deal still has to be done, but there is room for some optimism about a general solution to the problem of how proteins fold

review article

Bacterial behaviour

Howard C. Berg*

Bacteria swim by rotating their flagella They back up or choose new directions at random by changing the direction of the rotation The probability of such changes is biased by sensory reception. The bias depends on the way in which the intensity of the stimulus changes with time, so that the bacteria tend to swim up a gradient of attractant and down a gradient of repellent chemicals

BACTERIA move with intent, accumulating in regions which are hot or cold, light or dark, or of favourable chemical content Systematic studies of these phenomena began in the late nineteenth century[1-3], but only recently has it been clear that they can be understood in molecular detail[4] How do bacteria swim? How do they respond to changes in their environment? How do they detect and process sensory information?

Mechanism of propulsion

Bacteria swim by rotating thin helical filaments which project from one or more points on their surface (Fig 1) A filament is joined by a proximal hook to a rod and a set of rings embedded in the cell wall and the cytoplasmic membrane[5] The entire structure is called a flagellum If there are several flagella per cell, the filaments form bundles and move in unison The bundle both pushes (or pulls) and rotates the cell (Fig 1) Butschli[6] realised long ago that this is a natural consequence of the resistance of the medium to the motion of the flagella Whether a flagellum bends in a helical fashion, as in some eukaryotes, or rotates rigidly about its axis, as in bacteria, the effect is the same The forces involved are viscous rather than inertial[7,8], so much so that it is impossible for a bacterium to coast when the flagella stop moving, the cell comes to a standstill (in so far as Brownian motion will allow) within about a millionth of a body length The hydrodynamics of the propulsion of cells with polar flagella are well understood[9-11]

Bacterial and eukaryotic flagella are entirely different organelles The eukaryotic flagellum contains complex bending machinery[12,13], the bacterial flagellum does not[14-17] Its filament is only about 130 Å in diameter It is a self-assembling polymer of a single protein, flagellin, which has no known enzymatic activity Since the filament lacks local means for the interconversion of chemical and mechanical energy, it cannot actively bend How, then, does it do work on the medium in which it is suspended? It has been suggested that helical waves are driven from the base[16,18-20], but it is not clear that a filament propagating such waves could transfer the requisite amount of energy If the filament rotates as a whole, energy transfer is no longer a problem Rigid rotation was noted as a possibility by Stocker[14], proposed[21,22] and later abandoned[23,24] by Doetsch, then adopted[25-27] and later modified[28] by Jarosch Unfortunately, one cannot distinguish wave propagation from rigid rotation simply by looking at a cell[29] This is easily demonstrated with a model of a flagellum made by threading a rubber tube over a spiral wire[7,30] If the tube is fixed at one end and the wire is turned, the tube propagates a helical wave It looks as if it is rotating, but it is not If the wire is held fixed and the tube is turned, the tube revolves around the wire It looks as if

it is stationary, but it is not Unless one is close enough to see what is happening at the surface of the tube, it is impossible to tell that the tube is there

There is now a considerable body of evidence in favour of rigid rotation[31,32] The most dramatic has been obtained by tethering a cell with an abnormally long hook or a straight filament to a glass slide with anti-hook or anti-filament antibodies, when this is done, the cell body rotates, alternately clockwise and counter-clockwise[32] The cell is non-motile when free, but it spins several revolutions per second when the hook or the filament is linked to the glass Similar results have been obtained with anti-filament antibodies and cells with helical filaments[33] In each case, the body of the cell rotates, it does not merely precess or wobble It has been suggested that tethered cells may rotate because the end of the hook or filament in contact with the glass crawls around a circular path as it passes a helical wave (C R Calladine, personal communication) Experiments with polystyrene latex beads[32] are not subject to this argument When they are linked to cells with straight filaments, often several can be seen in tangential contact with (and revolving in synchrony about) a line projecting from the surface of the cell
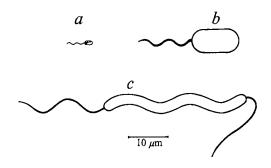
Fig. 1 Scale drawings of various bacteria *a*, *E coli* (or *Salmonella typhimurium*) About six filaments arise at random from the sides of the cell and form a bundle which appears near one pole The bundle pushes the cell When it changes its orientation, the cell goes off in a new direction *b*, *Chromatium okenii* About forty filaments arise at one pole When they change their direction of rotation, the cell backs up *c*, *Spirillum volutans*, shown swimming from left to right The body is helical About twenty-five filaments arise at each pole Those on the right are in the head configuration, those on the left in the tail configuration When they change their direction of rotation and flip over, the cell swims in the opposite direction In each case, the filaments rotate about 50 revolutions per second one way while the body of the cell rotates more slowly the other, translation occurs at speeds of about 20 cell diameters a second

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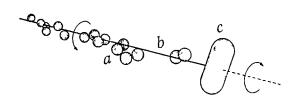
Fig 2 An experiment in which polystyrene latex beads (*a*) are linked to a straight filament (*b*) The filament is shown as a solid line, but it is invisible under phase contrast Most of the beads are linked directly to the filament, some are linked by means of other beads They revolve about the axis of the filament in one direction, while the cell body (*c*) rotates about this axis in the other There are no beats or undulations The beads remain fixed relative to one another Every now and again they slow down, stop, and start up in the opposite direction, the cell body changes its direction synchronously The entire assembly slowly diffuses through the medium The cell was derived from *E coli* AW405 (ref 34) by transduction with P1 from the donor W3110 (ref 32) The beads were linked to the filament by a method similar to that of Silverman and Simon[32]

(Fig 2) The flagella do not simply wind up and unwind mutants exist which rotate in one direction indefinitely[33]

If the flagella rotate, it is possible to understand (see ref 31) how cells with flagellar bundles are stopped by bivalent but not by univalent anti-filament antibodies[35,36], why cells with only one filament are inert to such bivalent antibodies[36], how peritrichously flagellated cells are stopped by flagellotropic phages[37,38], how cells with straight filaments may be able to form flagellar bundles[39], and finally, how cells with polyhooks but no filaments can counter-rotate when linked together with bivalent anti-hook antibodies[40] In making the first of these arguments, we noted that adjacent filaments in a bundle will stop when crosslinked by an antibody, because they can no longer rotate relative to one another Calladine[41] has argued that these crosslinks, if sufficiently rigid, could also prevent helical wave propagation by suppressing intra-filament "oscillatory lengthwise rubbing" This assumes that the filaments are inelastic, when, in fact, relatively little work would have to be done to deform them to meet the constraints on wave motion which such crosslinks impose (R A Anderson, personal communication)

A model for the flagellar rotary motor is shown in Fig 3 (see ref 42) The motor utilises as an energy source an intermediate in oxidative phosphorylation rather than ATP itself[43,44] It shares this property with a number of systems for active transport[45] Indeed, it could be driven by the translocation of ions through the M-ring which interact with fixed charges on the surface of the S-ring Some of the dynamic properties of the motor have been determined by following the rotation of tethered cells in the tracking microscope[42] The motor runs in either direction at about the same speed, it changes its direction abruptly, the motion can be remarkably smooth, there is little passive slip between the flagellum and the cell wall, and the amount of torque generated is impressive In *Escherichia coli*, some twenty genes are required for the complete assembly and function of the organelle[46,47], many are homologous to those found in *Salmonella*[48,49] One (*hag*) is the structural gene for flagellin, another (*flaE*) controls the length of the hook, a third (*flaI*) regulates the synthesis of the entire structure A normal *mot* product is required for the motor to rotate, normal *flaA* and *cheA* products are required for it to rotate both clockwise and counter-clockwise Most of the other genes have a non-flagellated mutant phenotype Only the *hag* gene product has been identified

Response to changes in environment

Bacteria have a limited repertoire of movements which vary depending on the size and shape of the cell and the distribution of the flagella (Fig 1) Most swim steadily in almost a straight line, then alter course abruptly *E coli* chooses a new direction

Fig. 3 A model for the flagellar rotary motor of a Gram-positive bacterium The rod is connected to the filament (not shown) by the hook, which serves as a universal joint[31] The torque is generated between the M-ring, which is mounted rigidly on the rod, and the S-ring, which is mounted on the wall The M-ring rotates freely in the cytoplasmic membrane The motor of a Gram-negative bacterium has an additional pair of rings[5] which probably serve as a bushing for the passage of the rod through the multilayered wall[42]

Fig 4 A projection of the track of a wild type *E coli* obtained with a microscope which automatically follows its motion in three dimensions[50,51] The points are 0 08 s apart The cell swims along as straight a path as rotational diffusion will allow (runs), stops and jiggles about (tumbles or twiddles), and then runs again The twiddles occur at random, on the average about once a second

almost at random (Fig 4)[50] *Chromatium okenii* backs up, moves bundle-first several body lengths, then swims forward once again[52], a manoeuvre which Engelmann[53] called a "shock reaction" Cells with a single polar flagellum, such as *Vibrio metchnikovii* (not shown) simply back up, they swim equally well with the flagellum in front or behind[54] *Spirillum volutans* flips its bundles from a head–tail to a tail–head configuration and swims off in the opposite direction[54,55] The probability of the occurrence of these events is biased by sensory reception It was thought for many years that bacteria back up or choose new directions at random on entering regions which are unfavourable that is, that the motor reflex is an avoidance response[53,56,57] This has not proved to be the case for *E coli*[50] When these

bacteria swim up a spatial gradient of an attractant (such as L-aspartate or L-serine), the probability that a twiddle will occur is smaller than it is in an isotropic solution, when they swim down such a gradient, the probability is nearly the same as it is in an isotropic solution Other parameters of the random walk remain constant The bacteria drift up the gradient because the runs are somewhat longer in the favourable direction The same asymmetry has been found in experiments in which a temporal rather than a spatial gradient is formed by enzymatic generation or destruction of an attractant As the concentration increases, the bacteria change direction less frequently, as it decreases, they swim as they do in the absence of a stimulus[58] Similar conclusions have been reached in experiments with *S typhimurium* in which solutions of an attractant at different concentrations are mixed[59,60] If the concentration of the attractant is suddenly increased, the bacteria swim smoothly for a relatively long period of time If it is suddenly decreased, they twiddle more frequently, but only for a relatively short period of time Attractants and repellents have opposite effects[61] The enzyme and the mixing experiments both show that the bacteria make temporal rather than spatial comparisons[56] In the enzyme experiments a response occurs even though the medium is isotropic In the mixing experiments the bacteria are exposed to both temporal and spatial inhomogeneities, they could measure the latter and time-average the results, but then the response would be the same on adding or diluting out the attractant

The motor reflex occurs when the flagellar 'motors' change their direction of rotation This was shown in early work on bacteria with polar flagella by Reichert[54], Buder[52], and Metzner[55] who, however, regarded the change as one in the direction of propagation of flagellar waves The use of the tethering technique has recently made a more direct proof possible for *E coli* Larsen *et al*[33] have shown that mutants of *E coli* that swim smoothly (that do not twiddle) rotate counter-clockwise when tethered to the top of a slide and viewed from above, while mutants that twiddle incessantly rotate clockwise Stimuli which cause the wild type to swim smoothly (or to twiddle) cause such cells, when tethered, to rotate counter-clockwise (or clockwise) Similar conclusions have been drawn from studies of *S typhimurium*[62] The bundle of a peritrichously-flagellated bacterium is able to push the cell but not to pull it, when the motors reverse, the bundle changes its orientation or comes apart[54,63,64], and the cell twiddles[33] The bundle of a bacterium with a polar flagellum is not subject to this instability, it remains intact, and the cell backs up *Chromatium okenii* completes the shock reaction by swimming forward once again, probably when its filaments tangle, a similar mechanism may terminate the twiddle[3,19] In so far as all bacteria able to respond to sensory stimuli are known to change directions spontaneously, it is likely that all bacteria have a "twiddle generator"[50] that reverses the flagellar motors[3] If so, the behaviour of a cell depends on the rate at which the generator fires in the absence of an external stimulus and on the extent to which the firing is enhanced or suppressed by sensory reception Parkinson[65] has concluded from an analysis of a large number of motile but non-chemotactic mutants of *E coli* that the machinery for twiddle generation and suppression involves only three gene products S-adenosyl methionine may be required for its function[4,66-68]

Chemoreception

A renaissance occurred in 1969 when Adler[69] proved that bacteria have specific chemoreceptors, that is, that they sense attractants *per se* There were suggestions of this in the early literature (for example, in Rothert's[56] experiments on competitive inhibition), but the idea never took hold Links[70], for example, proposed that the first (or only) step in the chain of events leading to the chemotactic response is a sudden decrease in the rate of utilisation of energy by the motor apparatus This hypothesis was developed further by Clayton[71,72], who applied it to taxis in *Rhodospirillum rubrum* Adler grew *E coli* on a chemically defined medium, washed and suspended the cells in a medium that supported motility but not growth[73], and then exposed the

cells to spatial gradients formed by the diffusion of attractants, from capillary tubes[74-76] By varying the initial concentrations of the attractant and counting the numbers of bacteria that swam into the tubes in a given period of time, he was able to establish a quantitative dose–response curve Using this method he showed that (1) chemicals that are extensively metabolised need not attract (even if they are the first metabolic products of chemicals that do attract), (2) chemicals that are not metabolised (because they are non-metabolisable *per se* or because the cells have lost the ability to metabolise them) may attract, (3) the response to an attractant is not generally blocked by the presence of structurally unrelated compounds (even if metabolisable), (4) structurally related compounds compete, (5) mutants exist which lack specific taxes (yet metabolise the attractant), and (6) transport of a chemical into the cell is neither necessary nor sufficient for it to attract

Chemoreceptors for amino acids[77], sugars[78], and a number of repellents[61,79] are now known The recognition components for D-galactose, D-ribose and maltose are shockable binding proteins[80] The role of the galactose binding protein in chemotaxis has been studied by Hazelbauer and Adler[80], that of the ribose binding protein by Aksamit and Koshland[81,82], and that of the maltose binding protein by Hazelbauer[83] Genetic analysis has shown that the binding protein for galactose interacts with other components that are specific either for taxis or for transport[84] The recognition components for D-glucose, D-fructose and D-mannitol are sugar-specific enzymes of the phosphotransferase system[78,85] Other components in these systems specific for taxis but not for transport remain to be identified For L-aspartate and D-galactose, twiddle suppression depends on the time rate of change of the fractional amount of chemoreceptor bound[58,86] For D-ribose the duration of the smooth response in a mixing experiment is proportional to the increment in the fractional amount of chemoreceptor bound[87] The cells monitor the status of their chemoreceptors, but the biochemical mechanisms are not known

Signal processing

Neurones integrate excitatory and inhibitory inputs electrically, responding in an all-or-none fashion by generating an action potential It is tempting to think of a bacterium in the same manner[88] and to suppose that the integration of different inputs[61,89] and the coupling between the receptors and the flagella occur through changes in the membrane potential[90] This is suggested, for example, by work on *S volutans* (Fig 1) Ordinarily, the flagellar bundles reverse synchronously[55], they seem to do so even when filmed at 48 frames per second[91] This implies that one end of the cell can signal the other in 10^{-2} s or less If the signalling were done by diffusion of a substance of low molecular weight, either through the cytoplasm of along the membrane, at least 1 s would be required[3] An electrical disturbance could be propagated much more rapidly The signalling can be impeded by the addition of large amounts of salt or blocked by partial deprivation of oxygen in the presence of a photodynamic dye[55] Other substances put the flagella in a head–head or a tail–tail configuration[55,91,92], but they may do so simply by forcing the motors to run in the same direction Some work has been done with drugs known to affect excitable membranes[91-95], but the results are hard to interpret In *E coli* relatively little is known about the mode of coupling between the chemoreceptors and the flagella[4,47] Mutants exist that fail to respond to compounds that use different chemoreceptors, such as D-ribose and D-galactose[84], or L-serine and certain repellents[79], L-aspartate and L-serine signal the flagella by means of different pathways[65] The system may prove to be complex Since both the receptors and the flagella reside in or interact with the cytoplasmic membrane, the latter's involvement is apparent

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articles

Experiments in catastrophe

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Two new theories of evolutionary instability are outlined When applied to highly optimised engineering structures, they predict that buckling strengths can be dramatically eroded by small unavoidable manufacturing imperfections this is confirmed by tests on stiffened panels of box-girder bridges A related analysis yields a spectacular picture of the hyperbolic-umbilic catastrophe

Two parallel and complementary contributions to our understanding of evolutionary instabilities have recently been presented The first is the widely acclaimed qualitative catastrophe theory of René Thom[1] which has been applied most fruitfully by Zeeman and others to problems in sociology, economics and theoretical biology[2].

The second is the quantitative general branching theory of Thompson and Hunt[3] which has had a major impact on our approach to the instability of elastic solids This is essentially a prototype bifurcation theory for slowly evolving systems in the spirit of Poincaré, and has wide applications across the mathe-

matical sciences in such areas as cosmology, hydrodynamics and crystallography

The two theories have been developed quite independently, but are remarkably similar in form and content, and Hunt and I have just completed a preliminary correlation between them[4] Cross fertilisation between the two disciplines promises to be most rewarding, and catastrophe theorists may be interested to find in ref 3 well developed perturbation schemes for the quantitative analysis of bifurcations and catastrophes, together with a wealth of catastrophe machines akin to that of Zeeman[5]

General theory

In their simplest form, the two theories relate to any system governed by a potential function $V(Q_i, \Lambda^j)$ where Q_i is a set of n state variables or generalised coordinates, and Λ^j is a set of k externally controlled parameters Stationary values of V with respect to the Q_i are supposed to be necessary and sufficient for equilibrium, while minimum values are supposed to be
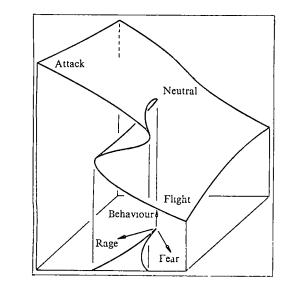
Fig. 1 Zeeman's illustration of the cusp catastrophe in the response of a dog subjected simultaneously to fear and rage

necessary and sufficient for stability The n equilibrium equations $\partial V/\partial Q_i = 0$ thus define surfaces[6] in the $k+n$ dimensional $(\Lambda - Q)$ space, while the vanishing of the stability determinant $| \partial^2 V/\partial Q_i \partial Q_j |$ identifies critical equilibrium states at which a loss of stability can be expected

The locus of critical states projected into the k-dimensional control space gives a catastrophe boundary which in elastic stability usually corresponds to an imperfection–sensitivity curve on a plot of the failure load against the imperfection magnitude Usually Λ^j is finally assumed to be slowly varying, and attention is focused on the steady evolution of an initially stable equilibrium solution which can be destroyed by a sudden dynamic snap from one of the critical equilibrium states

The two theories delineate the forms of instability that can occur by classifying the singularities that can arise in the catastrophe boundary, and a powerful result of topology allows Thom to identify seven "ordinary catastrophes" These are the only topologically stable, and therefore the only naturally occurring, forms that can arise in a control space of up to four dimensions ($k \leqslant 4$) no matter how many state variables Q_i there are in the system

This result has significance in developmental biology where the external control parameters of a cell might be its spatial coordinates (x,y,z) and the time t, the former influencing the cell through the spatially varying chemical environment Our conceptual catastrophe boundary now becomes a four-dimensional spatio-temporal boundary of behaviour[7]

Two applications

In the past, bifurcation and catastrophe theorists have tended to view their identical problems in rather different ways, and I can best illustrate this by means of two illustrative examples

An attractive illustration of qualitative catastrophe theory in an inexact science is Zeeman's demonstration of the cusp phenomenon in the psychological response of a dog or a man subjected simultaneously to feelings of fear and rage Here we must think of the intensities of fear and rage as being two controlled variables Λ^j under which we observe the behaviour of the dog as a single coordinate Q We thus expect to have a response surface which Zeeman[8] argues must have the form shown in Fig 1

Under low stimulus we see that the behaviour can vary continuously from flight to attack, but under high stimulus the dog's response varies discontinuously with jumps in behaviour at the fold-lines which project into a cusp in the plan view Clearly it is imperative for the animal that under intense pressure it should not respond in a neutral manner, even if

fear and rage are equally balanced, and correspondingly the inverted fold of the surface represents unrealisable unstable states

By contrast, a quantitative application[9] of the general bifurcation theory is sketched schematically in Fig 2 Here a square sheet of crystal is loaded by a direct force or stress σ_{11} and deforms accordingly into a rectangle with a direct elongation or strain ε_{11} At a certain load, however, a point of bifurcation C is encountered at which a symmetry-destroying shearing deformation ε_{12} develops and the crystal deforms unexpectedly into a parallelogram At this bifurcation load the directly stressed crystal would fail explosively in a homogeneous shearing mode A small constant shearing stress σ_{12} would clearly induce some shearing strain ε_{12} even before the bifurcation, so such a stress would act as an "imperfection" rounding off the bifurcation as shown

In this example the stresses σ_{11} and σ_{12} are to be thought of as two control parameters Λ^j whereas the strains ε_{11} and ε_{12} are to be identified as two state variables Q_i The first picture shows equilibrium paths in a three-dimensional graph, a significant projection of which is shown underneath on the left hand side The load carrying capacity as measured by the maximum direct stress $\sigma_{11}{}^M$ is severely reduced by the application of even a small σ_{12}, and the locus of critical points is finally projected into the control space to give a cusp-shaped failure stress locus of $\sigma_{11}{}^M$ as a function of $\sigma_{12}{}^M$
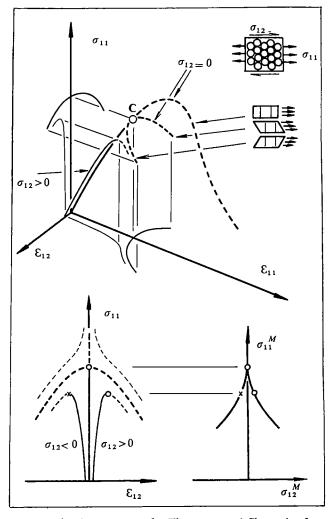
Fig. 2 The demonstration by Thompson and Shorrock of a bifurcation in the response of a mechanically stressed atomic lattice A close-packed crystal with Lennard–Jones interatomic potentials is considered and the primary path is shown to lose its stability in a symmetry-destroying unstable-symmetric point of bifurcation at C This gives rise to a cusp on the failure stress locus In an extension of this work we have shown that the crystal exhibits a hyperbolic-umbilic catastrophe in the three dimensional failure space of the planar stresses σ_{11}, σ_{12} and σ_{22}.
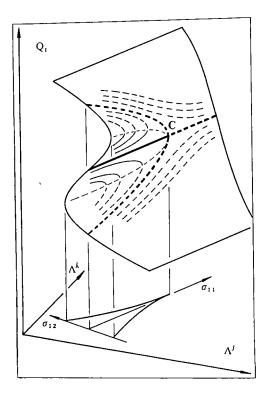
Fig. 3 Paths on an equilibrium surface demonstrating the equivalence between the unstable–symmetric point of bifurcation and the cusp catastrophe One distinction between the examples of Fig 1 and Fig 2 is that in the former the bulk of the surface is stable and the inverted fold is unstable while in the latter the situation is exactly reversed My stable– and unstable–symmetric points of bifurcation are each classified by Thom as a cusp catastrophe

Figure 1, with its emphasis on the whole equilibrium surface, is an example of Thom's Riemann–Hugoniot cusp catastrophe and Fig 2, with its emphasis on equilibrium paths, represents my unstable–symmetric point of bifurcation But in fact these are one and the same phenomenon, as is made clear in Fig 3 in which bifurcating paths are generated simply by slicing the folded equilibrium surface at constant σ_{12}

This cusp, or distinct symmetrical point of bifurcation, which generates a two-thirds power law cusp in Λ space, is by far the most common branching phenomenon and can be found throughout the mathematical sciences, one significant example arising in the instability of rotating stellar masses[10,11]
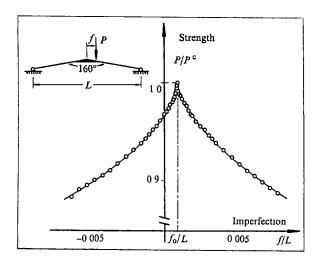
Elastic arch

This ubiquitous cusp was first studied experimentally by Roorda at University College, London, and one of his many tests is summarised in Fig 4 Here a shallow steel arch pinned to rigid abutments is loaded, nominally at its crown, by a load P which is progressively offset by a small distance f to simulate an asymmetric manufacturing imperfection The behaviour of the arch is elastic throughout, and failure is associated with the dynamic snapping of the arch through asymmetric configurations into an inverted state

In this example the load P and the offset f are to be regarded as two controlled parameters while the in-plane rotation of the crown, θ, can be viewed as a single generalised coordinate measuring the asymmetric deformation The equilibrium paths
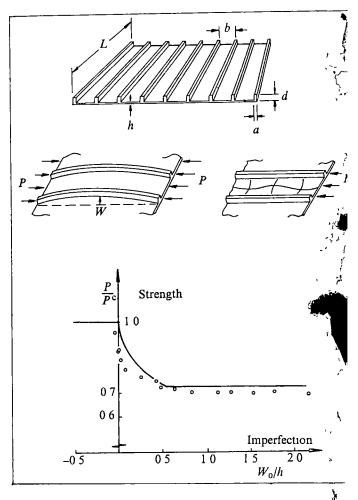
Fig. 5 The sensitivity of the strength of a stiffened panel to manufacturing imperfections W_0 in the overall form The model panel tested was of epoxy plastic with dimensions $h = 0 75$ mm, $b = 57 5$ mm, $a = 4 9$ mm and $d = 10$ mm The graph corresponds to a section through the hyperbolic-umbilic catastrophe of Fig 6 This test is one of a continuing series conducted by A C Walker

of the family of arches generated by varying f have the general form of the unstable–symmetric point of bifurcation shown in the lower graphs of Fig 2 with P replacing σ_{11}, f replacing σ_{12} and θ replacing ε_{12}

The right-hand-side cusp is of particular interest and now represents an imperfection–sensitivity curve on a plot of the failure load P as a function of the simulated imperfection magnitude f The curve determined experimentally is shown in Fig 4 This follows the predicted two-thirds power law, and shows a dramatic deterioration of strength away from the optimum 'design' at $f = f_0$ at which the critical offset f_0 is just cancelling out the unavoidable manufacturing errors in the test specimen (see also ref 3)

Fig. 4 Test by Roorda on a model arch, showing a deterioration of strength with the controlled asymmetry
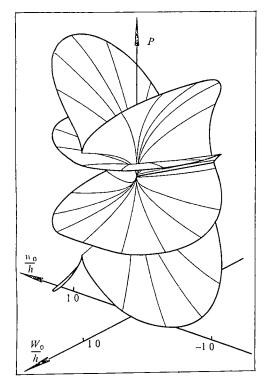
Fig 6 The hyperbolic-umbilic catastrophe determined by Hunt (unpublished) in the theoretical response of a stiffened elastic panel The lower region of the surface gives the panel strength as a function of the overall and local manufacturing imperfections[4]

Highly optimised structures

I have just presented an example of an optimum design which is extremely sensitive to unavoidable manufacturing imperfec-tions, and it is my thesis[3,12] that as higher and higher degrees of optimisation are sought, so the severity both of the failure and of the associated imperfection-sensitivity will rise Indeed us is likely to be a feature of any highly optimised system, whether in the field of engineering structures or not

As an illustration of this I consider the elastic buckling of an optimised stiffened panel similar to those employed in box-girder bridges The model panel of Fig 5 is compressed by an axial load P, parallel to the stiffeners, under which the specimen may deflect like a simple column in an overall mode with amplitude W as shown on the left, or may alternatively ripple between the stiffeners in a local mode with amplitude w as indicated on the right

The much used, but potentially dangerous concept of simultaneous mode design[3,12] states that the structure will be optimal when these two failure modes can develop simul-taneously, and I have chosen the length of the panel so that this condition is closely realised A simulated initial deflection of amplitude W_0 in the overall mode represents a manufacturing imperfection, and we[13] have obtained the imperfection-sensitivity curve shown, failure being characterised by a sudden dynamic snap in the range of material elasticity A dangerous erosion of strength with positive imperfection amplitude is indicated, and the experimental points are seen to be in quite good agreement with the curves of a simplified *ad hoc* theoretical treatment

This treatment and more details of the tests are to be re-ported elsewhere[13] They show that highly optimised plate assemblages must be expected to have the severe imperfection-sensitivity characteristics previously associated only with the notorious buckling behaviour of thin shells, a conclusion that will undoubtedly carry over into the plastic range

Hyperbolic-umbilic catastrophe

A similar stiffened elastic panel containing additionally a local imperfection of amplitude w_0 has been analysed more rigorously by V Tvergaard, and his asymptotic equations corresponding to our homeoclinal point of bifurcation have been solved in detail by G W Hunt (unpublished) to give the complete imperfection-sensitivity surface of Fig 6, the lower region of which gives the panel strength as a highly-peaked function of the two imperfection amplitudes W_0 and w_0 This dramatic computed picture has been identified by D Chilling-worth of Southampton University as a hyperbolic-umbilic catastrophe, and this seems to be the first known practical example of this predicted form

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Evolution of Precambrian crust from strontium isotopic evidence

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The strontium isotope evidence places constraints on the evolution of Precambrian crust Contrary to popular belief, sialic crust cannot, it seems, be "reworked" on a grand scale

MANY geologists believe intuitively that Archaean and post-Archaean gneisses, the ultimate origin of which is not recog-nisable in the field, are somehow derived from "much older" sialic (that is, continental igneous, sedimentary or metamor-phic) crust A particularly popular hypothesis is that continental,

sialic crust can somehow be "reworked" repeatedly on a grand scale (The term reworked is here used in the sense of partial or complete melting leading to mobilisation and reconstitution as an essentially new rock and not in the frequently used, but incorrect, sense of isochemical recrystallisation and/or defor-mation) These views are not compatible with the results of some recently published Sr isotopic data on Archaean and Proterozoic gneisses, and greenstone belt volcanic rocks with associated later granodioritic or tonalitic plutons

The views presented below are an extension of those of

Spooner and Fairbairn[1], who reported low, if somewhat imprecisely determined, initial $^{87}Sr/^{86}Sr$ ratios for several suites of Precambrian pyroxene granulite gneisses In particular, they favoured an igneous origin for such rocks, involving transfer into the crust of a mantle-derived magma of approximately andesitic composition Spooner and Fairbairn suggest that the part that reaches the upper crust will crystallise as normal extrusive and intrusive rocks, but any part that is trapped in the lower crust at about 10^5 KPa will cool slowly to a minimum of about 500 °C and crystallise directly into plagioclase–pyroxene assemblages, characteristic of the granulite facies In this connection, igneous rocks with magmatic features characteristic of fractional crystallisation but with typical granulite facies mineralogy are well known from such areas as Lofoten–Vesteraalen, north Norway, where large quantities of mangeritic and charnockitic magmas were intruded at depths of 20–25 km and at 900–1,000 °C (ref 2)

Geochemistry and isotope data

The geochemistry of major elements of many pyroxene granulite and amphibolite facies gneisses (see, for example, ref 3) suggests strong affinity with igneous rocks of calc-alkaline character, in spite of the characteristic depletion in certain incompatible trace elements such as U, which shows up so particularly well in the present unradiogenic Pb isotope ratios in many high-grade gneisses[4][5] This depletion occurred within the uncertainty of the measured whole rock Pb/Pb age and has been interpreted as an essentially metamorphic phenomenon[4,5] The highest grade gneisses sometimes also show strong synmetamorphic Rb depletion, leading to present unradiogenic Sr isotope ratios[1,6]

Table 1 is a summary of recently reported Rb—Sr whole rock ages and initial $^{87}Sr/^{86}Sr$ ratios of some major Precambrian gneiss complexes Also shown are measurements on basaltic-to-andesitic greenstone belt volcanics and associated (either cross-cutting or presumed later) calc-alkaline plutons from two areas Almost all initial $^{87}Sr/^{86}Sr$ ratios are in close agreement in the general range 0 700–0 702, and fall rather close to the value presumed to be characteristic of the upper mantle for the corresponding age[7]

Figure 1 shows averaged $^{87}Sr/^{86}Sr$ growth lines, calculated from the mean of the measured Rb/Sr values for each individual rock unit in the publications cited in Table 1 The slopes of the lines are proportional to the Rb/Sr ratios, which are mostly within the range 0 2–0 4 for the gneisses and granites listed in Table 1, although each rock unit exhibits considerable dispersion in Rb–Sr values The data for Greenland, Scotland and Rhodesia are plotted in relation to a hypothetically linear upper mantle growth line extending from 0 699 to 0 703 in 4,600 Myr, corresponding to a Rb/Sr ratio of about 0 02 (It is not yet known for certain whether this line is strictly linear, or slightly convex upwards implying nonlinear evolution of upper mantle Sr^7) The positions of all initial ratios fall almost on, or slightly above, the linear upper mantle growth line, and demonstrate beyond any reasonable doubt that later rock units cannot have been derived by the reworking of earlier ones of the type exposed in any given region The same conclusion applies to the North American data, which are not plotted because of the large grouping of ages at around 2,700 Myr and the relative uncertainty in the values for the Minnesota River Valley gneiss

Implications for evolutionary models

The measured ages and initial $^{87}Sr/^{86}Sr$ ratios for unaltered greenstone belt volcanics and later plutons almost certainly relate to the time of crystallisation For a gneiss complex, they could relate to the time of regional metamorphism In that case, linear extrapolation of any given $^{87}Sr/^{86}Sr$ growth line back to the linear upper mantle growth line yields an upper age limit for the precursors of the gneiss complex In most cases this does not exceed about 100 Myr, and in some cases it is less than 50 Myr Since high-grade metamorphism may cause a decrease in Rb/Sr ratios, the first part of the $^{87}Sr/^{86}Sr$ growth line could have been significantly steeper, so that the age difference between formation of the precursors and their metamorphism would be correspondingly reduced A nonlinear upper mantle growth line (convex upwards) would, of course, have much the same effect

Several major Archaean and Proterozoic gneiss complexes, as well as greenstone belt volcanics and associated later plutons, are predominantly juvenile additions to the continental crust at, or close to, the measured age, implying continental growth on a major scale during the Precambrian (Table 1) Furthermore, the

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Table 1 Rb–Sr whole rock ages and initial $^{87}Sr/^{86}Sr$ ratios of Precambrian rock units

Rock unit and area	Age (Myr)*	Initial $^{87}Sr/^{86}Sr$
Greenland and Scotland		
Amîtsoq gneiss, Godthaab and Isua areas, West Greenland[23]	3,750 ± 50	0 7010 ± 0 0005
Nûk Gneiss, Godthaab, West Greenland[24]	3,040 ± 50	0 7026 ± 0 0004
"Nûk" Gneiss, Sermilik, West Greenland†	3,110 ± 80	0 7010 ± 0 0010
Ketilidian granite gneiss, South-west Greenland[25]	1,890 ± 90	0 7022 ± 0 0010
	1,780 ± 20	0 7032 ± 0 0005
Grey gneiss, Outer Hebrides, Scotland[26]	2,690 ± 140	0 7014 ± 0 0007
Rhodesia		
Basement granite gneiss[27,28]	3,600 ± 200	0 701 ± 0 001
Gwenoro gneiss[28]	2,780 ± 30	0 7011 ± 0 0001
Maliyami formation (Main greenstone belt)[28]	2,720 ± 70	0 7010 ± 0 0002
Sesombi tonalite pluton[28]	2,690 ± 70	0 7008 ± 0 0004
North America		
a Greenstone belt volcanics		
Michipicoten, Ontario[29]	2,600 – 2,700	0 7018 ± 0 0002
Coutchiching and Keewatin, Minnesota and Ontario[30,31]	2,600 – 2,700	0 7010 ± 0 0004
Yellowknife, NWT[12]	2,625 ± 160	0 7022 ± 0 0023
b Gneisses and granites		
Minnesota River Valley Gneiss[32]	approx 3800 (?)	approx 0 700 (?)
Northern Light, Saganaga, Icarus, Grants Range, Algoman, Vermilion, Minnesota–Ontario border[30,33][34]	2,600 – 2,700	0 7010 ± 0 0005
Yellowknife, NWT[12]	2,640 ± 40	0 7011 ± 0 0008
Chibougamou, Quebec[35]	2,740 ± 230	0 7009 ± 0 0005
	2,680 ± 80	0 7005 ± 0 0002
Wind River Range, Wyoming[36]	2,630 ± 20	0 702 ± 0 001
Twilight Gneiss, Colarado[37]	1,805 ± 35	0 7015 ± 0 0004

*^{87}Rb decay constant 1 39 × 10^{-11} yr^{-1}
†R J Pankhurst and S M, unpublished

interval between the time of extraction of juvenile igneous material from the upper mantle (and/or subducted oceanic lithosphere) and the time of production of the regional metamorphic characteristics of the derived gneiss complex may be less than 50–100 Myr, which falls within the analytical uncertainty of most age measurements of Archaean and Proterozoic rocks It is possible that during such an "accretion" event, major amounts of juvenile igneous material broadly equivalent in composition to the island arc tholeiite series and, in particular, to the calc-alkaline series, were produced at depth in an ancient proto-island arc regime[8-11] Some of this material came directly to the surface of the pre-existing continental or oceanic crust where it gave rise to the volcanic assemblage characteristic of greenstone belts The remainder crystallised at depth as the hypabyssal and plutonic equivalents of the high level rocks The
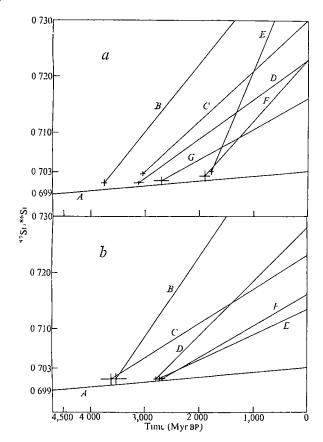
Fig. 1 Average $^{87}Sr/^{86}Sr$ growth lines for *a*, Greenland and Scotland *A*, upper mantle growth line, *B*, Amitsoq gneiss, Godthaab and Isua areas[23], *C*, Nûk gneiss, Godthaab area[24], *D*, "Nûk" gneiss, Sermilik area (Oxford unpublished data), *E,F*, Ketilidian granite gneisses, south-west Greenland[25], *G*, Grey gneiss, Outer Hebrides, Scotland[26] *b*, Rhodesia *A*, upper mantle growth line, *B*, Mushandike granite gneiss[27], *C*, Mashaba granite gneiss[28], *D*, Gwenoro migmatitic gneiss[28], *E*, Maliyami formation greenstone belt[28], *F*, Sesombi tonalite pluton (cross-cutting the Maliyami formation)[28]

volcanic association is probably the early extrusive expression of the subcrustal processes which later led to the intrusion of calc-alkaline plutons into the deforming volcanic-sedimentary pile, as already suggested for the Yellowknife Province by Green and Baadsgaard[12] At depth, the plutonic material slowly crystallises with igneous or metamorphic mineral assemblages, simultaneously undergoing geochemical and petrological differentiation to give a compositionally layered lower crust[13-17], in which the major and minor components of granite (*sensu lato*) migrate upwards to form rocks of the calc-alkaline plutonic association, leaving behind a thick residue of depleted granulite facies rocks at greater depth The resulting crustal layering is gradational and highly complex in detail Under these plutonic conditions the

conventional distinction between what is "igneous" and what is "metamorphic" is no longer clearcut Furthermore, intermediate zones of variably depleted calc-alkaline amphibolite facies gneisses must also be quantitatively important Most of the gneisses in Table 1 are actually of this type

In some cases, upward migrating volcanic and plutonic materials penetrate much older continental, sialic crust (as in Rhodesia) which could have formed in much the same way during an earlier continental accretion episode But this older sialic crust, where present, may contribute little or no "reworked" material (Fig 1), although it may well undergo renewed metamorphism and tectonism (as in the Godthaab area of West Greenland), as well as providing xenoliths and enclaves of many different types in the younger volcanic and plutonic rocks But it seems, in principle, unlikely that relatively low density, continental sialic crust can be buried or subducted sufficiently deeply for really large scale reworking of basement gneisses of the type postulated by many geologists (see, for example, refs 18 and 19) True reworking of older sialic crust may, of course, happen on a limited scale above ancient and modern subduction zones, and it is quite possible that in certain tectonic regimes such as in orogenic belts formed in sites of continental collision with resulting major crustal thickening, sialic crust can be truly reworked on a moderate scale[20] Localised partial melting of ancient, sialic crust may also occur in the zone of heating above large bodies of basic igneous magma introduced into high crustal levels, as for example in the Tertiary igneous province of north-west Scotland[21] All of these complicating factors which involve partial melting of, and assimilation with, ancient sialic crust are clearly exhibited by the complexities of initial Sr isotopic ratios in many relatively high level granites and related rocks of mainly Phanerozoic age[7]

The mechanisms of continental growth and crustal evolution implied by plate tectonics may well be adequate to explain the evolution of major Precambrian crustal units, although there is no final proof At any rate, they are certainly compatible with the Sr isotopic evidence The sequence of events comprises. plate formation—plate separation (ocean floor spreading) —plate convergence—subduction—partial melting of basic lithosphere and/or upper mantle—extraction and emplacement of basaltic and calc-alkaline volcanic and plutonic rock types— accretion of continental crust—geochemical and petrological differentiation of new continental crust The Sr isotopic evidence suggests that this sequence can be relegated for any given cratonic suite, which exhibits close grouping of rock-forming ages and low initial $^{87}Sr/^{86}Sr$ ratios, into a continuous or semicontinuous "super-event" not exceeding 50–100 Myr in duration This value is of the same order as found at present between the most recently formed complementary ocean ridges, island arcs and continental margins

The major conclusions drawn from the Sr isotopic evidence are, of course, unaffected by the particular model chosen for continental growth, whether plate-tectonic or otherwise But for the examples cited here, the process of repeated reworking of significantly older sialic crust with anything like normal crustal Rb/Sr ratios must be rejected

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letters to nature

Energy spectrum of diffuse component of cosmic soft γ rays

IN this paper we present new measurements on the diffuse component of cosmic soft gamma rays in the energy range 0 1–4 MeV obtained with a balloon-borne telescope These measurements show that the energy spectrum gradually steepens from $E^{-2\,3}$ to $E^{-2\,8}$ as the energy increases from 100 to 500 keV and becomes less steep thereafter, consistent with the presence of a hump in the MeV region

Previous observations[1–5] of the diffuse gamma ray spectrum obtained both at balloon altitudes and with detectors on board spacecrafts are subject to criticism In the spacecraft observations[6,7], the counting rate in the detectors resulting from radioactivity induced by cosmic ray interactions is comparable to that of the cosmic component of gamma rays at energies above 1 MeV and the corrections for this effect are not free from ambiguity In balloon observations the procedure for deducing the background contribution from secondary cosmic rays by linearly extrapolating the growth curve is not justified for omnidirectional detectors[8–10] These difficulties can be circumvented by employing the "active shutter method" described previously[11]

This experiment is an improved version of the scheme with which a well defined directional response for the gamma ray detection has been achieved previously The central NaI (Tl) counter of 7 5 cm diameter and 7 5 cm thickness is surrounded by a cylindrical NaI (Tl) counter of 10 cm wall thickness and 30 cm height The bottom hole is covered by a CsI (Tl) crystal, 7.5 cm in diameter and 7 5 cm thick A shutter counter made of NaI (Tl) of 7 5 cm diameter opens and closes the entrance aperture at the top for alternate periods of one minute By taking the difference between the counting rates of the open and closed modes, the background caused by the radiation outside the entrance aperture is eliminated The angular response of the detector for this difference is nearly triangular with FWHM of about 40°, as determined by using gamma ray sources [57]Co (122 keV), [137]Cs (660 keV), [60]Co (1 7, 1 33 MeV) and [24]Na (1.37, 2 75 MeV) The geometrical factor at 1 2 MeV is 23 cm^2 sr. The detection threshold of the guard counters and the shutter counter are set at 15 keV The response of the detector to neutrons has been measured using an Am–Be source, the counting rate of the telescope at the ceiling has a negligible contribution from atmospheric neutrons

The gamma ray detector was flown from Hyderabad, India on April 13, 1974 The balloon stayed at the ceiling altitude of 4,300 Pa (4 3 mbar) for about two hours and then gradually

descended to 6,800 Pa over the next three hours The pulse heights measured in the central counter were analysed into 128 channels and telemetred event by event along with the indication of coincidence with the shutter and/or the guard counters The counting rates of the guard counters were also monitored In-flight calibrations of the central and shutter counters revealed showed that the functioning of the apparatus was stable throughout the flight

The pulse height distributions observed at the ceiling altitude are shown in Fig 1 for the open and the closed modes of operation of the telescope Their difference, also shown in the same figure, represents the pulse height distribution for the 'forward gamma rays' To determine the gamma ray intensity at the top of the atmosphere the following functional form for
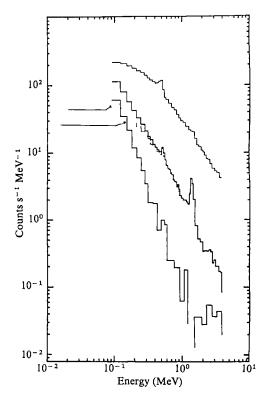
Fig. 1 Observed pulse height distributions of the central counter at the ceiling altitude *a*, Anti-off, open, *b*, anti-on, open, *c*, closed, *d*, difference (open–closed) "Anti-off" indicates guard counter pulses disregarded
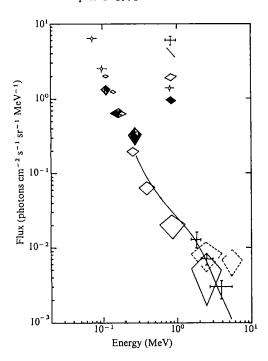
Fig 2 Energy spectrum of soft gamma rays The present result is indicated by open diamonds Dashed diamonds represent the fluxes not corrected for the electron contribution ◆ and O, Our previous balloon observations, +, Schonfelder and Lichti (unpublished, solid line, ref 5)

the growth curve was assumed for altitudes higher than 50 g cm^{-2}

$$C(z) = I_0 F(z) + I_b z$$

where $C(z)$ is the counting rate at an altitude z (in g cm^{-2}), I_0 is the intensity of primary gamma rays, $F(z)$ is the attenuation function of primary gamma rays in air and I_b is the constant The last term is the intensity of the atmospheric component, and its assumed linear z-dependence for the narrow aperture telescope used in the present experiment is supported by Monte Carlo calculations[8-10] $F(z)$ is taken from a Monte Carlo calculation by Horstman and Horstman-Moretti[12]

The energy loss spectrum has been transformed to photon spectrum by the use of response functions obtained for different energies using radio isotopes A comment is necessary for the data above 1 MeV Since the entrance aperture is not protected against charged particles, re-entrant albedo electrons are suspected to give an appreciable contribution in this energy region This has been estimated using the observed electron spectrum[9] The spectrum of the gamma rays thus derived with the utmost correction of the electrons is shown by open diamonds, whereas the flux values without the corrections are shown by dashed diamonds in Fig 2 The effect of the electrons is estimated to be negligible below 1 MeV

The present results are compared in the same figure with our earlier results The filled diamonds are those obtained from a balloon flight made at Hyderabad in 1972 with a similar detector equipped with a plastic guard counter and the open circles are based on a balloon observation from Sanriku, Japan (also in 1972) with a passive collimator and a passive shutter There is good agreement between the various results in the overlapping energy regions The spacecraft results by Trombka et al[5] and the balloon results by Schonfelder and Lichti[13] are also shown in Fig 2 These are in general agreement with our results although their flux values are somewhat higher

Our principal conclusion is that the spectrum gradually steepens in the energy range 100–500 keV and then tends to flatten in the MeV region consistent with a hump around 1 MeV Single power spectra can fit the data points only with confidence

levels lower than 0 001 Detailed description of the experiment and data analysis will be published elsewhere[13]

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Neutrino processes and QSOs

PECULIAR jets of matter emanate from some galactic nuclei and QSOs, including the galaxy M87 and the QSO 3C273 It is generally believed that these jets are matter ejected from the central regions of the object[1] As a result of non-conservation of parity in weak interactions, the neutrino processes associated with gravitational collapse of a galactic nucleus in the presence of a strong magnetic field can lead to ejection of matter from the nucleus in one direction I suggest that the jets observed in M87 and 3C273 are the result of this mechanism

The importance of neutrino processes in gravitational collapse depends on the very large mean free path of neutrinos even in extremely dense matter, which enables them to carry away the gravitational potential energy released in the collapse Because of conservation of magnetic flux associated with the "frozen in" magnetic field, a collapsing object can acquire an intense magnetic field[2] In the presence of an intense magnetic field a certain fraction of neutrons in the object will be aligned with spins parallel to the magnetic field, in weak processes involving aligned neutrons the neutrino emission is asymmetrical as a result of non-conservation of parity in weak interactions So the linear momentum carried away by neutrinos is non-zero and must be compensated by a change in linear momentum of the system The degree spin alignment of neutrons depends on temperature T and the strength of the magnetic field H An almost complete spin alignment is possible if $\mu H > kT$ (μ is the magnetic moment of the neutron, k the Boltzmann constant) For a temperature of the order of 10^8 K, this condition is satisfied if $H \sim 10^{15}$ gauss Optical and radio observations probably rule out the existence of magnetic fields of this strength in the outer regions of a galactic nucleus or a QSO But the occurrence of magnetic fields of this strength in the central collapsing regions of the nucleus, where the neutrino processes are important, cannot be ruled out Ginsburg[3] has shown that the magnetic field of a collapsing object

can increase without limit There are also arguments which suggest that highly collapsed matter is 'ferromagnetic' with spins of neutrons aligned in one direction[4] If we assume almost complete spin alignment, the neutrino angular distribution function $W(\theta)$ is proportional to $(1+\alpha\cos\theta)$, where θ is measured from the direction of the magnetic field (for nuclear β decay, α is of the order 0 4) If N neutrinos are emitted with average energy E a simple calculation gives the momentum ΔP carried away by neutrinos as

$$\Delta P = (2NE\alpha/3c)n \qquad (1)$$

where n is unit vector in the direction of the magnetic field Thus if a mass δM is converted into neutrinos, by setting $NE = \delta Mc^2$ in equation (1) the momentum carried away by neutrinos is

$$\Delta P = 2/3\,(\delta Mc\alpha)n \qquad (2)$$

So to conserve linear momentum the object should either recoil or eject matter in the opposite direction For the mechanism to be effective a large fraction of the material of the nucleus must be in the form of neutrons free to decay This requirement can probably be realised In the central regions of a collapsing object matter should exist predominantly in the form of neutrons since at high pressures it is thermodynamically more favourable for protons to capture electrons The presence of few per cent of electrons will ensure that neutrons are stable against β decay But there is no reason to believe that the Fermi energy of these electrons will remain the same throughout the collapse A sudden decrease in Fermi energy will allow neutrons to decay The quantity δM in equation (2) can be a considerable fraction of the mass of the object Since α is not very different from unity, large amounts of matter can be ejected in a direction opposite to n with relativistic speeds The energy released during neutron decay could temporarily convert the contraction into an expansion Repetition of this process will also occur but in every contraction phase ejection of matter will take place in the same direction because of the presence of the "frozen in" magnetic field

The jets associated with most objects have condensations along their length indicating that several ejections have taken place in the same direction This is in agreement with the mechanism I propose It is interesting that no other mechanism could easily explain the occurrence of discrete emissions from the nucleus in the same direction If the jet formation is a magnetohydrodynamic effect there is no reason why the discrete ejections should not occur with equal probability in a direction parallel as well as anti-parallel to the magnetic axis

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Time markers in interstellar communication

THE feasibility of interstellar radio communication using existing facilities has been established in principle[1] We can communicate with extraterrestrial civilisations whose technological powers are no greater than ours by concentrating radiated power and receiver sensitivity in a narrow band of frequencies and a narrow field of view

Frequency selection is a problem that seems to be tractable

because there are clearly defined markers, spectral lines of hydrogen and hydroxyl, in the low noise region of the radio spectrum[1,2] Although there may be no single optimum frequency, the number of frequencies that must be considered seems to be quite small

The number of stars that can be considered as possible homes for communicative civilisations, on the other hand, is very large Recent studies[3-6] agree that the chances of two civilisations establishing contact are exceedingly small in the absence of time markers that will tell the two civilisations when to search for one another Here we point out that suitable time markers do exist for binary stars They are the turning points of the stellar orbits, periastron and apastron

Single star civilisations would logically transmit signals to binaries at the observation of periastron and apastron Binary star civilisations would scan single stars at a time $2T$ after periastron and apastron, where $2T$ is twice the signal propagation time between the stars The length of scan need be no longer than twice the uncertainty in T Contact signals from different stars would arrive at different times at the binary system because of differences in the value of T for each candidate This strategy would enable a binary star civilisation to complete a search of nearby stars with a small number of telescopes after only a few orbital revolutions

The same strategy would be followed for transmissions from one binary civilisation to another Contact signals would be sent at the time of observed periastron and apastron of the receiving civilisation Contact signals would be sought at a time $2T$ after local periastron and apastron

The strategy for a binary civilisation attempting to contact a single star civilisation depends on whether the signals are transmitted isotropically or non-isotropically Signals transmitted isotropically from a binary at its own periastron and apastron will arrive at each target star at the observation of periastron and apastron On the other hand, with non-isotropic transmission, individual transmissions must be made to all target stars, necessitating very short transmissions or a very large number of transmitters A solution to this problem would be to delay transmissions by a time known to both civilisations, that is T This would preserve the time markers but spread the calls out in time

It may, on the other hand, be preferable to send the contact signals to single stars at the times when the angular distance between the binary stars as seen from the target star is either a maximum or a minimum These times are known to both civilisations Because they depend on the orientation of the orbit with respect to the line joining transmitter and receiver the times are different for each target star Simultaneous calls to many candidates are therefore avoided by this strategy

Although the optimum strategy may not be immediately apparent, these considerations limit the possibilities to the point where the search in time is no more difficult than the search in frequency For any advantage to be gained from these strategies, however, it is necessary that the number of reasonable contact times, multiplied by the duration of the search period required to accomodate errors in distance and in orbital elements, should not exceed the period of the binary star These strategies therefore require accurate knowledge of stellar parallaxes The strategy of listening for contact signals from binary stars at the times of observed turning points of the orbit or at the times of maximum angular separation does not require knowledge of the distance on the part of the receiving civilisation, however, so it might be advantageous for us The transmitting civilisation does need to know the distance in order to determine when to listen for replies, but binary star civilisations should be able to measure stellar parallaxes more accurately than we can because of the long baseline provided by the binary orbit

There is no shortage of binary star systems that may be the homes of communicative civilisations Of the 100 nearest stars at least 40 are visual binaries, and a total of more than 50,000 visual binaries are known[7] The probability of the existence of habitable planets in binary star systems has been examined by

Dole[8,9] and Huang[10,11] There seems to be no reason why visual binaries should not have habitable planets, provided one or both of the stars is of suitable spectral class and provided their ecospheres have radii considerably smaller than the separation at periastron

In the Worley Catalog of Visual Binary Orbits[12] there are 536 systems with known orbits and periastron dates Of these we have determined that about 300 systems may have habitable planets about one or both components The number of stars in these systems that may support habitable planets exceeds 500 As an illustration of the possibilities we have prepared a partial list of opportunities for the detection of signals from civilisations associated with visual binaries that we judge to be habitable Considering only systems closer than 300 light yr we find 34 opportunities between 1975 0 and 1980 0

If there are communicative civilisations associated with any of the nearby binary stars it should occur to them, as it has to us, to use the orbital motions of their stars as an indicator of when to send and when to look for contact signals There seems to be a reasonably small number of possibilities These considerations could lead to a non-random search programme with some chance of success They could also provide a practical basis on which to initiate our own transmission of contact signals

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Upper atmospheric thermal structure in Antarctica

THE mesospheric wind results derived for atmospheric altitudes between 50 and 90 km from meteorological rocket flights carried out at Molodezhnaya station (67° 40′ S, 45° 51′ E) in Antarctica have been discussed in ref 1 The rapid shifts in both zonal and meridional components of the winds during May to July indicated a sudden 'explosive' change in temperature distribution in the upper mesosphere over Antarctica in the winter regime I have investigated this phenomenon and extended the study down to the stratosphere, the results are discussed here The meridional and the zonal temperature gradients at altitudes of 65, 70 and 75 km, derived from the thermal wind equations using the corresponding upper wind and temperature results, have also been studied Atmospheric temperatures up to an altitude of about 80 km were also measured and the accidental root mean square errors in the determinations were as follows in the altitude region 60 to 80 km it does not exceed

7 to 10 °C, at 50 km it is 5 °C, and below 40 km the error is less than 3 °C

Time-height cross sections of the mesospheric temperatures in the region 50 to 80 km are plotted using the conventional interpolation method in Fig 1 Meridional and zonal temperature gradients are derived for altitudes of 65, 70 and 75 km from the corresponding upper wind and temperature results using the thermal wind equations

$$\frac{\partial T}{\partial y} = \frac{fT}{g}\left(\frac{\partial u}{\partial z} - \frac{u}{T}\frac{\partial T}{\partial z}\right)$$

$$\frac{\partial T}{\partial x} = \frac{-fT}{g}\left(\frac{\partial v}{\partial z} - \frac{v}{T}\frac{\partial T}{\partial z}\right)$$

where f is the coriolis parameter, g the acceleration due to gravity, T the absolute temperature at a particular level, x, y, z the eastward, northward and vertical axes, u, v the zonal and meridional components of winds with west and south positive, respectively The horizontal temperature gradients are given in Table 1

Time sections of the upper atmospheric temperatures showing annual variations at the altitudes 30, 50 and 70 km as derived from the successful 1972 rocket soundings are plotted in Fig 2 Corresponding upper atmospheric warming and cooling of more than 15 °C over periods of less than 15 d are given in Table 2 for 5-km altitude intervals during the winter period May to August and early spring

Figure 1 and Table 1 are confined to the mesosphere only, whereas Fig 2 and Table 2 extend the study to the stratosphere Figure 1 shows that in the southern summer months January–February the mesospheric temperatures in the region 50 to 80 km ranged from about 10 to −110 °C, with the summer maximum reached by about January 5 in the lower mesosphere and by about January 26 in the upper mesosphere The disruption of the mesospheric winter regime by a period of stormy winds[1] is clearly shown in Fig 1 In the third week of May and the first week of July the upper mesosphere was subjected to a warming of more than 30 °C in less than a week The temperature distribution returned to normal by early September, but was again disrupted in about the third week

Table 1 shows that the horizontal temperature gradients at 65 km ranged from −37 to 10 °C per 5° latitude with a maximum on September 6 At 70 km the range was −31 to 30 °C with the maximum values on June 28 and May 17, whereas the gradients at 75 km ranged from −14 to 21 °C per 5° latitude with the maxima on September 6 and May 17, respectively Larger gradients were found from May to July, indicating a disruption by stormy winds having large wind shears of about 10×10^{-3} s^{-1} to 30×10^{-3} s^{-1} A similar disruption occurred in September The negative meridional temperature gradients represent a decrease of the zonal winds with altitude (the easterlies increase and the westerlies decrease with height) and the negative zonal temperature gradients represent an increase of the meridional winds with altitude (the northerlies decrease and the southerlies increase with height) On May 17 at 70 km the meridional temperature gradient was 30 4, suggesting a disruption by strong easterlies which decreased with height

Curve C in Fig 2 shows that at 30 km the stratospheric temperatures in Antarctica fall as the Sun sets, and that the minimum −75 °C is reached in June A slow temperature rise begins as the Sun returns, but this becomes much more marked after early September when the Sun's radiation reaches the lower strata Curve B (50 km, around the boundary of the stratosphere and the mesosphere) shows a warming of 23 °C during the second week of May followed by an equal cooling during the third week During the second week of July there was a mid-winter stratospheric warming of 30 °C The temperatures at 50 km ranged from a maximum of 12 °C in early

Table 1 Meridional and zonal temperature gradients at Molodezhnaya, Antactica, in 1972

Date	65 km Temperature gradient*		70 km Temperature gradient*		75 km Temperature gradient*	
	Meridional	Zonal	Meridonal	Zonal	Meridonal	Zonal
January 5	0 3	−2 1	−6 3	−0 8	−1 7	3 1
January 19	− 2 0	−0 6	0 3	−6 5	2 5	1 4
January 26	0 8	3 0	−1 7	2 6	10 6	−1 7
February 16	− 9.4	−0 3	3 3	3 5	−5 0	−5 9
May 3	− 9 3	−4 0	−5 0	17 8	−1 0	−2 1
May 17	−20 2	−8 1	30 4	−0 5	−4 8	20 9
June 28	5 2	2 3	−31 3	10 5	−7 1	5 5
July 5	−13 4	−7 8	−20 9	6 9	−7 5	−1 0
July 19	−10 4	−3 7	8 2	5 6	9 1	1 9
September 6	−36 7	9 9	−1 1	−1 7	9 1	−14 1
October 4	− 0 6	−3 8	−5 4	5 4	2 7	−2 2
December 20	0 1	0 7	4 2	−3 4	−0 2	3 7

* °C per 5° latitude

December to a minimum of −45 °C at the end of May Curve *A* (70 km) reveals sudden 'explosive' changes in the upper mesospheric temperature distribution, particularly during the winter period May to July During the third week of May, a warming of 26 °C occurred, and in midwinter there was a warming of 31 °C during the first week of July followed by a 'sudden cooling' of 53 °C during the second week The temperatures at 70 km show a distinct maximum of −12 °C during the first week of July, after which the temperature declines irregularly, attaining a minimum of −93 °C in mid-November Again in September there was a disruption with a warming of 20 °C followed by a cooling of 33 °C after which the temperature distribution returned to the normal summer regime

Table 2 reveals that in May there was a significant warming both in the upper stratosphere and the mesosphere with a 'sudden warming' of 49 °C at 73 km from May 17 to 24 The warming was followed by a cooling of about 30 °C in the lower mesosphere From June 28 to July 5, a warming of 37 °C was detected at 65 km The upper stratosphere and the lower mesosphere were subjected to a warming from July 5 to 19, with a maximum of 38 °C at 55 km, whereas the upper mesosphere underwent a 'sudden cooling' which had a maximum value of 55 °C at 65 km from July 5 to 12 In August and

Fig. 1 Time–height cross section of the mesospheric temperatures (°C) at Molodezhnaya, Antarctica in 1972 Dashed lines, extrapolated data Arrows above the abscissa show the dates on which the data were obtained
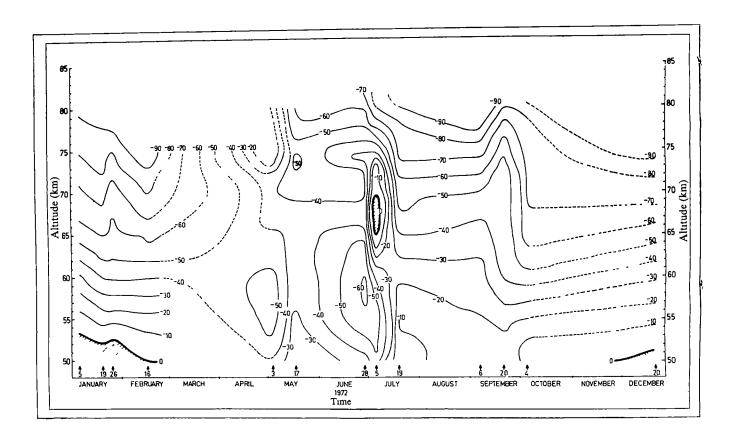
Table 2 Upper atmospheric warming and cooling of greater than 15 °C over periods less than 15 d at Molodezhnaya, Antarctica, in 1972

Altitude (km)	May Warming	May Cooling	June-July Warming	July Warming	July Cooling	July-August Warming	July-August Cooling	August Warming	August Cooling	August-September Warming	September Warming	September Cooling
35				22* (5–12)†				27 (9–16)			38 (6–20)	
40	18 (10–17)			29 (5–12)		21 (26–2)		32 (9–16)	−26 (2–9)		39 (6–20)	−20 (20–27)
45	21 (10–17)	−18 (3–10)		19 (5–12)					−22 (16–23)		17 (13–20)	
50	26 (3–17)	−32 (17–24)		31 (5–19)					−26 (9–23)	21 (23–6)		
55	24 (10–17)	−27 (17–24)		38 (5–19)			−23 (26–2)			19 (23–6)		−26 (13–27)
60	22 (10–17)	−28 (17–31)	19 (28–5)	20 (12–19)			−26 (26–2)	18 (20–23)		15 (23–6)		−27 (20–27)
65	36 (20–24)		37 (28–5)		−55 (5–12)		−18 (26–2)	22 (20–23)			19 (13–20)	−22 (20–27)
70	26 (20–24)		31 (28–5)		−53 (5–12)						20 (13–20)	
73	49 (17–24)				−48 (5–12)			16 (6–20)			16 (13–20)	
75	29 (17–24)				−25 (22–26)			16 (6–20)				
80	36 (17–20)	−43 (20–24)			−39 (22–26)	22 (26–2)		18 (6–20)				

*°C
†Dates

September the warming in the mesosphere was less than 27 °C, while the stratosphere at 40 km experienced a cooling of 26 °C from August 2 to 9, followed by a warming of 32 °C from August 9 to 16 At 40 km there was another warming of 39 °C from September 6 to 20, which was followed by a cooling of 20 °C from September 20 to 27
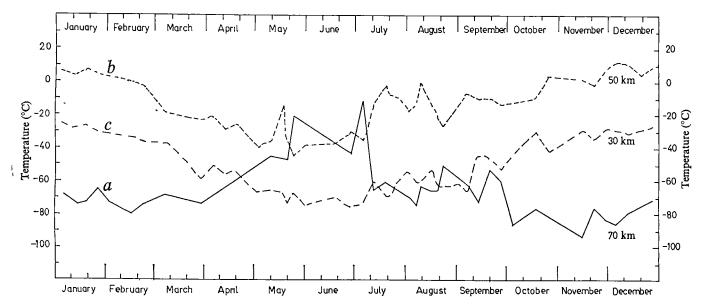
This investigation of the Antarctic upper atmosphere indicates that the most active period in south polar regions is the winter and the early spring it is marked by large disruptions in the wind and thermal structure The rapid shifts in both zonal and meridional components of the upper atmospheric winds, particularly during the winter period May to July, were accompanied by sudden changes in the temperature distribution as revealed by warming and cooling in Fig 2

Figure 2 shows that the stratospheric warming in May propagated upwards and was followed by the mesospheric warming The stratospheric warming in July followed the

mesospheric warming, indicating downward propagation of the disturbance which started above 70 km This leads to the intuitive conclusion that the polar winter warmings may be caused both by an increase in the supply of energy in the form of a vertical flux of geopotential energy consisting of very long waves, and by radiative and photochemical processes taking place in the upper atmosphere During September, when the winter westerlies changed to the summer easterlies, the upper atmosphere was again disrupted with a warming of 39 °C at 40 km which is attributed to the increase in available heat brought about by the return of sunlight It is thus concluded that sizeable perturbations may occur in the upper atmosphere over Antarctica during the winter regime

Fig. 2 Time section of annual variation of the upper atmospheric temperatures (°C) for the altitudes 30, 50 and 70 km at Molodezhnaya, Antarctica, in 1972

part in the expedition, and giving me the privilege of being the first Indian explorer of Antarctica, which was made possible by the late Professor Vikram A Sarabhai I thank Professor P R Pisharoty for guidance and discussions I also thank my parents for inspiration

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Phlogopite stability and the $^{87}Sr/^{86}Sr$ step in basalts along the Reykjanes Ridge

THE relatively sharp step in the $^{87}Sr/^{86}Sr$ ratio within basalts midway along the Reykjanes Ridge may reflect the breakdown of titaniferous phlogopite during variable fusion of an essentially homogeneous phlogopite-lherzolite upper mantle, extending beneath both Iceland and the submarine parts of the Mid-Atlantic Ridge

Schilling[1] detected substantial variation in the concentrations of several elements within basalts collected along the Reykjanes Ridge, south of Iceland To explain this he proposed two magma sources, a primordial hot mantle plume, relatively enriched in large-ion lithophile (LIL) trace elements and radiogenic Sr and Pb isotopes, rising beneath Iceland, and relatively LIL-depleted upper mantle within the low velocity layer beneath the Mid-Atlantic Ridge Mixing of these sources and/or their partial melt products was postulated to produce the chemical variation observed along the Reykjanes Ridge Subsequent work[2,3] showed that the transition from Icelandic (0 70304) to Mid-Atlantic Ridge (0 70270) values for the $^{87}Sr/^{86}Sr$ ratio in basalts was not by a smooth gradation but by a step confined to a zone 200–250 km south of the Reykjanes Peninsula Several other trace elements behave similarly Critics of the two-mantle-source model[4,5] have emphasised that most Icelandic basalts (some of which approach tholeiitic andesite compositions) are very strongly fractionated relative to the compositions of magmas that could conceivably have been generated by partial fusion of any postulated upper mantle material The rare-earth element (REE) patterns reported by Schilling[1] may be fitted adequately to a model invoking variable partial fusion of a single source[5]

The proposals of Schilling's critics do not account for two crucial observations first, the varying Sr and Pb isotopic ratios in the lavas, second, the high rate of magma production in Iceland relative to deep-sea sections of the Mid-Atlantic Ridge, as evidenced by thickening of the crust beneath the former[6] We are reluctant as yet to accept the two-mantle-source model because it is essentially defeatist It may eventually be proved that the two-mantle-source model is correct, but meanwhile the single-mantle alternative should continue to be considered We concentrate here on $^{87}Sr/^{86}Sr$ variation in the basalts, perhaps the most persuasive evidence for the two-mantle-source model, and attempt to show that it is not irreconcilable with a single mantle composition

$^{87}Sr/^{86}Sr$ in basalts from the transition zone between the relatively isotopically-homogeneous lava groups of Iceland and the Mid-Atlantic Ridge shows a good correlation with the water depth at each collection site (Fig 1 and ref 3) This reflects local topographical irregularities in the Reykjanes Ridge and is not merely a result of the overall deepening of the Atlantic south of
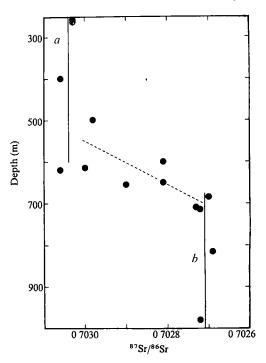
Fig 1 $^{87}Sr/^{86}Sr$ of Reykjanes Ridge basalts as a function of the water depth from which they were dredged[1,3] The Iceland and Mid-Atlantic Ridge average values[3] of $^{87}Sr/^{86}Sr$ extend for a very much greater range of water depths (~ 0–2,500 m) along the axis of the North Atlantic than that covered by this diagram *a*, Mean $^{87}Sr/^{86}Sr$ for Iceland, *b*, mean for Mid-Atlantic Ridge

Iceland, there is little correlation of $^{87}Sr/^{86}Sr$ between the points in the transition zone in Fig 1 and their distance from the tip of the Reykjanes Peninsula Hart *et al* [3] attribute this relationship to dynamic interfingering between their two postulated mantle types This proposal seems improbable when the high postulated viscosity of the upper mantle (~ 10^{22} poise) at appropriate pressure and temperature[7] is compared with the small scale of topographical[8] and isotopic variation in the transition zone, lavas with distinctively different $^{87}Sr/^{86}Sr$ ratios are found as little as 2 km apart[3]

There are two alternative explanations of the $^{87}Sr/^{86}Sr$–depth relationship, both coming from discoveries[9,10] that volcanism on the Mid-Atlantic Ridge is essentially confined to the axis of the structure and that, as the European and American plates separate at a constant rate (on the spatial and temporal scale considered here), the thickness of the ridge lava pile, monitored by water depth, is a direct measure of the rate of magma production beneath each ridge segment It follows that

(1) If the system is constrained by assuming constant mantle circulation rates beneath both topographic highs and lows on the Reykjanes Ridge, a greater amount of mantle partial fusion must have occurred beneath the highs than beneath the lows during basalt genesis So the relative ^{87}Sr-enhancement of lavas from the highs may reflect the thermal breakdown of an ^{87}Sr-rich mineral in the upper mantle This phase would remain in the residuum during production of the magmas which give rise to the Mid-Atlantic Ridge basalts and only melt during more intense fusion beneath Iceland and the northern Reykjanes Ridge
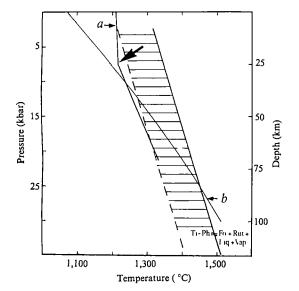
(2) If the driving force behind the movement of separating lithospheric plates is independent of mantle turnover beneath mid-oceanic ridges[11], the mantle passively inflowing between them as they part could move at very different rates in different localities If mantle fusion is related to its flow rate through heat generation resulting from viscous dissipation[7], the topographical heights on the Reykjanes Ridge may simply reflect zones of relatively rapid mantle creep beneath them The degree of partial

melting involved in the production of each magma batch might in this case be the same beneath both the Mid-Atlantic Ridge and Iceland Indeed, it may be even less beneath the latter, if enhanced kneading within fast-flowing mantle causes a positive correlation between flow rate and efficiency of magma extraction during partial fusion[12] In this case it is possible that magma generation takes place at greater depths beneath Iceland than the Mid-Atlantic Ridge The $^{87}Sr/^{86}Sr$ step in Reykjanes Ridge basalts may mark the breakdown of an ^{87}Sr-rich mantle mineral with increasing pressure northwards at the site of partial fusion If the Reykjanes Ridge crust is extremely weak, as seems probable from what is known of the strength of thin hot crust elsewhere[13], its topographical irregularities may give rise to small local pressure differences (< 50 bar, see Fig 1) at the level of partial melting below The $^{87}Sr/^{86}Sr$–depth correlation within the transition zone in Fig 1 would then reflect passage of this ^{87}Sr-rich mantle phase in and out of its stability field along irregularities in the isobar marking its maximum stability at depth

Is there a likely upper mantle phase with the chemistry and *PT* stability to satisfy any parts of the genetic models discussed above ? We suggest that phlogopite is the mineral responsible for the $^{87}Sr/^{86}Sr$ step It has been postulated frequently as a minor phase in the upper mantle[14-19] and the suggestion has been made in two recent papers[20,21] that fusion of phlogopite might be important in controlling K, Rb, Ba and ^{87}Sr contents of Icelandic and Mid-Atlantic Ridge basalts But neither discusses the *PT* stability of phlogopite relative to other likely phases in the upper mantle

Experimental studies of the stability of synthetic Ti-free phlogopite[18,19,22] have indicated that, if undersaturated with water at moderate pressures, it would probably be stable up to the solidus of a peridotite upper mantle but would enter the first fraction of liquid formed But the phlogopite that occurs as a primary phase in generally-accepted xenolithic samples of the upper mantle, such as garnet–lherzolite blocks in rapidly erupted volcanic rocks[16], frequently has a substantial Ti content (< 9% TiO_2) Synthetic Ti-phlogopite [$K_2Mg_4TiAl_2Si_6O_{20}$ $(OH)_4$] has a considerably greater thermal stability at all in-

vestigated pressures than its Ti-free analogue[23] Even making allowance for the presence of other mantle phases (extrapolated from the Ti-free phlogopite data), it seems probable (Fig 2) that Ti-phlogopite would remain in the residuum after fusion of a considerable fraction of a peridotite upper mantle at pressures below about 10 kbar Only at higher pressures would Ti-phlogopite enter near-solidus melts

The data of Fig 2 may be applied to upper mantle fusion beneath Iceland and the Mid-Atlantic Ridge, if the geothermal gradients in these areas are known The latter are not easily extrapolated from surface heat-flow measurements in regions of active volcanism, because of magma movement near the surface, and geothermal water circulation Experimental petrology is an alternative way of approaching the problem Kushiro ard Thompson[24] studied the melting behaviour of Mid-Atlan Ridge lavas as a function of pressure One of the most Mg-r ᴗh of the phenocryst-poor basalts (MgO = 9 17%) as yet known from the Ridge shows liquidus coprecipitation of three phases (olivine + pigeonite + plagioclase) at 7 5 kbar The simplest interpretation of this phenomenon[24] is that the basalt formed by fusion of a plagioclase–lherzolite upper mantle at 7 5 kbar and was erupted without significant further modification Fe/Mg of phases near liquidus in the basalt indicate a residual mantle, after Mid-Atlantic Ridge basalt extraction, with Mg/(Mg+Fe) ~ 0 85, in accordance with estimates from other volcanic areas[11,25] Acceptance of a primary magma hypothesis for this lava allows calculation of a Mid-Atlantic Ridge geothermal gradient, fitted to the liquidus temperature (1,215 °C) at the four-phase point The result of 45 °C km^{-1} overall corresponds well with previous estimates by other methods[26,27] Unfortunately, there are no comparable experimental data available as yet to allow calculation of Icelandic geotherms

If the geotherm below the Mid-Atlantic Ridge reaches 1,215 °C at about 7 5 kbar, it is appreciably above the anhydrous solidus at that pressure of either a pyrolite[28] or a Mg/(Mg + Fe) = 0 85 lherzolite[29] upper mantle (Fig 2) But it is approximately at, or slightly below, the extrapolated stability limit of Ti-phlogopite

The two models for the $^{87}Sr/^{86}Sr$ step in basalts from the Reykjanes Ridge may now be reassessed

(1) If the degree of mantle partial fusion during magma genesis increases northwards beneath the Reykjanes Ridge, then the $^{87}Sr/^{86}Sr$ step in Fig 1 and its correlation with thickness of lava pile may reflect the isobaric breakdown of titaniferous phlogopite in the source of the basalts This proposal leads to the surprising conclusion that the relative enrichment of Icelandic basalts in the 'incompatible' elements K, Rb, Cs, Ti, Ba and ^{87}Sr (ref 28) may not be because they originate as lesser fractions of mantle fusion than Mid-Atlantic Ridge basalts At first sight this seems highly improbable, because light REE and P are also concentrated in the Icelandic lavas But phlogopite can be relatively enriched in the light rare-earth elements[30] and its breakdown could contribute these elements to the melt Furthermore, concomitant REE and P variation may reflect the stability relations of apatite in the upper mantle The behaviour of this phase during partial fusion may be as complex as that postulated here for phlogopite Until this is known, or until data are published for LIL elements that are not concentrated in apatite or phlogopite, it seems premature to reject this model for the chemical variation in the Reykjanes Ridge basalts

(2) If the depth of the partial fusion zone increases northwards, the $^{87}Sr/^{86}Sr$ step beneath the Reykjanes Ridge may mark the intersection of this zone with the Ti-phlogopite breakdown curve In this model the degree of partial melting could decrease towards Iceland, so that apatite might then be envisaged as entering the first melt fraction An attractive feature of this alternative is apparent when it is applied to the general problem of the chemical differences between mid-oceanic ridge and ocean island lavas, of which the Iceland–Mid-Atlantic Ridge system is just a facet Ocean islands forming away from the crests of mid-oceanic ridges are composed, in general, of lavas less saturated in Si than those erupted at spreading axes[17,31,32] The available

Fig 2 *P–T* diagram correlating the following data anhydrous solidus of pyrolite[28], a possible model composition for the upper mantle, anhydrous liquidus of Mid-Atlantic Ridge magnesian basalt T-87 (ref 24), upper limit of thermal stability, in conditions free from vapour, of titaniferous phlogopite and extrapolation of this to stability in the presence of other likely upper mantle minerals[23] , "T-87 Geotherm" (see text), — — —, extrapolated stability, *a*, T-87 liquidus, *b*, anhydrous pyrolite solidus Arrow marks four-phase point

experimental data[29,33] indicate that partial fusion of a lherzolite upper mantle at less than 8 kbar, as postulated earlier for Mid-Atlantic Ridge basalts, could not yield nepheline-normative magmas, however low the degree of partial melting The latter have probably been generated at pressures above about 15 kbar (refs 25, 29, 33)

The largest potential weakness in all the foregoing argument concerns [87]Sr/[86]Sr equilibration amongst mantle phases This must be assumed not to occur either before or during partial fusion, if enrichment of magmas with [87]Sr is to be linked with phlogopite stability Most [87]Sr in phlogopite-lherzolite mantle will be generated from Rb contained in the phlogopite Isotopic studies of the minerals within peridotite xenoliths from sub-continental upper mantle sources indicate approximate equilibrium[34] in phlogopite-bearing garnet-lherzolites and disequilibrium within spinel-lherzolites[35,36] The relevance of data derived from material originating in continental parts of lithospheric plates to conditions in the sub-oceanic upper mantle requires further study At present we strongly support the geochemically based model of O'Nions and Pankhurst[21], which postulates lack of isotopic equilibration amongst the solid phases of a homogeneous phlogopite-lherzolite upper mantle, both before and during its partial fusion at sites of magma genesis, as the cause of [87]Sr/[86]Sr (amongst other) differences between Mid-Atlantic Ridge and Atlantic Ocean Island lavas

I L Gibson, M J O'Hara and P Suddaby made detailed constructive criticisms of the manuscript M F J F and H-U S received support from the Deutsche Forschungsgemeinschaft

Note added in proof Our attention has been drawn to a paper by M J O'Hara, M J Saunders and E L P Mercy (*Phys Chem Earth*, in the press) which contains ideas very similar to ours on the postulated major role of upper mantle phlogopite in controlling [87]Sr contents of basalts

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Palaeogeotherms and the diopside–enstatite solvus

THE precise composition of the solid solutions that develop between diopside and enstatite has provided a basis for estimates of the temperature of equilibration of mineral assemblages from peridotites We report here results of a reinvestigation of these relationships, which demonstrate that the range of solid solutions formed at high temperatures is less than previously believed, and that it is dependent on both pressure and silica activity The stable existence of an iron-free pigeonite has not been confirmed

Garnet-lherzolite xenoliths in kimberlite are widely accepted as xenoliths of the upper mantle They contain olivine, garnet and enstatite coexisting with a clinopyroxene They can be divided into two groups, in one the clinopyroxene is calcic and in the other it is subcalcic The distribution of analyses of the clinopyroxenes is bimodal[1]

Temperatures and pressures of equilibration of the mineral assemblages in individual nodules from the xenoliths have been estimated[2] The palaeogeotherms which existed in the upper mantle immediately before incorporation and transport of the xenoliths by kimberlite eruptions have been derived from data from large numbers of individual xenoliths[2] These geotherms show an abrupt rise in the rate of increase of temperature with depth, coincident with the change in clinopyroxene composition from calcic to subcalcic diopside

The temperature estimates were based on a comparison of the Ca/Ca+Mg ratio of the diopside coexisting with enstatite in the natural rocks, with the Ca/Ca+Mg ratio of diopside coexisting with enstatite in the system $CaMgSi_2O_6-Mg_2Si_2O_6$, as determined by experiments at 30 kbar (ref 3) The procedure assumed this relationship to be independent of pressure The results on the synthetic system indicate that enstatite is in equilibrium with a single clinopyroxene phase over the whole temperature range investigated and that the composition of that diopside solid solution changes continuously with temperature The rate of change of the diopside composition did not, however, vary regularly, and reached a maximum at temperatures close to 1,450 °C (ref 3) A study[4] of the same system at 20 kbar reported an abrupt change of the clinopyroxene coexisting with enstatite at this same temperature, from a calcic diopside at lower temperatures to a pigeonite at higher temperatures Above 1,450 °C a further solvus was reported between the pigeonite and a more calcic diopside The composition–temperature field occupied by a single clinopyroxene solid solution in the results reported[3] at 30 kbar is thus occupied by three fields, of clinopyroxene A (diopside), clinopyroxene B (pigeonite) and clinopyroxene A plus clinopyroxene B in the results reported[4] at 20 kbar

The possibility that the abrupt change from diopside to pigeonite in equilibrium with enstatite might have a bearing on the abrupt change from calcic to subcalcic diopside in the xenoliths from kimberlite, and on the change in slope of the inferred palaeogeotherms, prompted this reinvestigation of the clinopyroxene limb of the solvus at 20 and 30 kbar, the results show that pigeonite is not an equilibrium product in this system at those pressures The position of the equilibrium diopside limb of the solvus at high temperatures is very different from that observed by Davis and Boyd[3] Coexisting diopside and enstatite solid solutions do not have the same Si/Ca+Mg ratio, that is, the join $CaMgSi_2O_6-Mg_2Si_2O_6$ cuts across the tie lines linking coexisting diopside and enstatite solid solutions which depart, in opposite senses, from the ideal 1 1 ratio of Si/Ca+Mg The position of the diopside limb is, moreover, pressure dependent

The discrepancies between these and previous results are predominantly a function of the nature of the starting material Results obtained from glass starting materials yield metastable
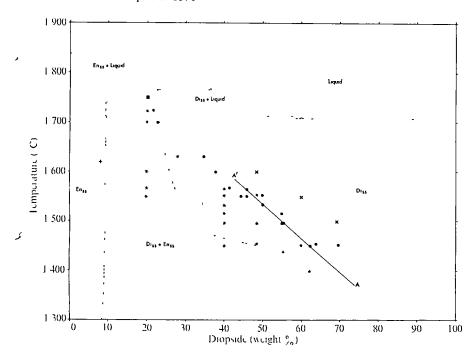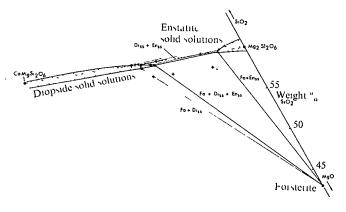
Fig 1 Results obtained at 30 kbar from compositions lying along the diopside–enstatite join, shown in relation to the interpretation by Davis and Boyd[3] (-----) A–A' = The diopside limb of the diopside–enstatite solvus obtained from runs on the gel composition $Di_{40}En_{60}$ weight % Results from gel starting materials ★, bulk composition and temperature of runs which crystallised to a solid solution of diopside plus enstatite, ●, composition of diopside in equilibrium with enstatite, from X-ray measurements, ×, homogeneous diopside solid solution obtained, +, enstatite solid solution obtained, ■, enstatite solid solution plus liquid, ○, liquid, ▲, composition of diopside solid solutions coexisting with enstatite solid solution crystallised from the glass of bulk composition $Di_{40}En_{60}$ weight %, , solidus in present work SS, solid solution (in all figures)

products When very fine-grained crystallised gels are used as starting material they react readily and completely to yield the equilibrium products

A high pressure piston-cylinder apparatus (see ref 5) was used in these experiments Temperatures were measured using Pt/Pt 13 rhodium thermocouples with no correction for pressure effects The starting materials were finely ground gels which had been recrystallised at 900 °C Runs were made both on homogeneous gels ($Di_{40}En_{60}$, $Di_{50}En_{50}$) and on mechanical mixtures of pure diopside and enstatite gels (bulk compositions $Di_{20}En_{80}$, $En_{28}En_{72}$, $Di_{40}En_{60}$, $Di_{60}En_{40}$, $Di_{69}En_{31}$) All compositions are quoted in weight % A glass of composition $Di_{40}En_{60}$ was also used for more direct comparison with the earlier works[3,4] which had used glass starting materials All charges were dried at 1,050 °C for 1 h and sealed in platinum capsules Runs were initially taken to 5 kbar above the desired pressure, the excess pressure being bled off when the required temperature was reached In the temperature ranges 1,450–1,500 °C, 1,500–1,565 °C and above 1,600 °C, the durations of the runs were at least 4 h, 2 h and 0 5 h, respectively, except where stated At 1,450 °C no change in the clinopyroxene composition occurred when experiments were run for longer

Fig 2 Part of the system $MgO–SiO_2–CaO$(weight %) at 30 kbar and 1,550 °C, showing bulk compositions of charges (+), and measured clinopyroxene compositions (●), in relation to inferred variation of SiO_2 in the diopside solid solution field (the extent of which along the join $CaMgSi_2O_6–Mg_2SiO_4$ is taken from refs 6 and 7) Tie lines to inferred enstatite compositions (▲) are shown intersecting the $CaMgSi_2O_6–Mg_2Si_2O_6$ join

than 1 5 h, and it was therefore assumed that equilibrium had been attained in all experiments used for drawing up the diopside solvus

Products were identified by optical and X-ray diffraction methods Attempts made to analyse clinopyroxenes using the electron microprobe failed because of the small grain size (< 5 μm) of the crystals, it was not possible to focus the electron beam on a unique grain, and interference from adjacent crystals produced a wide range of clinopyroxene compositions (for example, $Ca/Ca+Mg = 0 10–0 25$ in one of the coarser grained charges) The X-ray diffraction technique used chromium radiation in an evacuated chamber Compositions of clinopyroxenes were estimated from the relationship reported by Davis and Boyd[3] which our study verified as valid between 20 and 69 weight % diopside Clinopyroxene compositions could be obtained with a precision of ±1% diopside (99 7% confidence limits) The precision is rather less good for charges containing large amounts of enstatite This technique is capable of resolving two clinopyroxenes differing by as little as 10 weight % of diopside Clinopyroxenes which crystallised in equilibrium with enstatite at 30 kbar from the starting composition $Di_{40}En_{60}$ lay along the curve A–A' (Fig 1) irrespective of whether or not the homogeneous gel or the mixed diopside–enstatite gel was used Clinopyroxenes which crystallised from starting compositions $Di_{20}En_{80}$ and $Di_{28}En_{72}$ lay away from this curve by up to 4 weight % diopside towards enstatite, clinopyroxenes from starting compositions $Di_{50}En_{50}$ and $Di_{60}En_{40}$ lay up to 7 weight % diopside away from curve A–A[1] towards pure diopside When 10 weight % of forsterite was added to each of the gels $Di_{20}En_{80}$ and $Di_{40}En_{60}$ at 1,550 °C and 1,565 °C, constant clinopyroxene compositions of $Di_{41 5}En_{58 5}$ and $Di_{42}En_{58}$, respectively, were generated from both compositions This indicates that in detail the parageneses have the form shown in Fig 2

Experiments using glass of composition $Di_{40}En_{60}$ as starting material yielded homogeneous clinopyroxene above 1,440 °C as required by Davis and Boyd's[3] phase diagram which was obtained from runs on glass charges At 1,440 °C and 1,400 °C this starting material crystallised to enstatite and clinopyroxenes which lay exactly on the diopside limb of Davis and Boyd's[3] solvus

Runs on the homogeneous gel $Di_{40}En_{60}$ at 1,450 °C yielded enstatite and a clinopyroxene Di_{56} after 10 s and a progressively more magnesian clinopyroxene (up to Di_{50}) in longer runs of up to 30 min This trend towards the solvus found by Davis
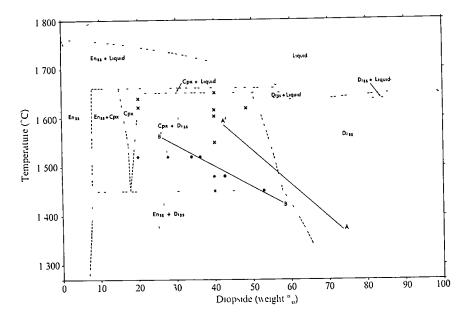
Fig 3 Results obtained in this work at 20 kbar from compositions lying along the diopside–enstatite join, shown in relation to the interpretation by Kushiro[4] B–B'= Approximate position of the diopside limb of the diopside–enstatite solvus obtained from experiments on gel starting materials A–A', and all symbols as in Fig Cpx, clinopyroxene

and Boyd[3] was reversed in longer runs, as the clinopyroxene changed towards the solvus A–A' (Di_{60} after 50 min, Di_{63} after 90 min), indicating the latter to represent the closer approach to equilibrium The effects may indicate that the exchange of Ca and Mg between the two pyroxenes proceeds more rapidly than the adjustments in silica content implied in Fig 2

Figure 3 shows the results at 20 kbar from compositions lying on the diopside–enstatite join in relation to phase boundaries obtained[4] using glass charges We found that enstatite coexists with a diopside lying close to the curve B–B' (Fig 3) The boundary B–B' at 20 kbar is significantly displaced towards En relative to A–A' at 30 kbar, but this pressure effect is for charges which were not saturated with forsterite The pressure effect is, however, also significant in forsterite saturated charges Forsterite (10 weight %) was added to the mixed gel of bulk composition $Di_{20}En_{80}$ at 1,520 °C The clinopyroxene which crystallised in equilibrium with enstatite and forsterite had the composition $Di_{37.5}En_{62.5}$ When the temperature of the forsterite bearing assemblage at 30 kbar is raised by 30 °C the clinopyroxene becomes 4 weight % richer in diopside The effect of pressure on the diopside solvus (20–30 kbar) at 1,535±15 °C is to increase the amount of apparent diopside by 0 8 weight % per kbar in forsterite saturated assemblages The effect in the stoichiometric join $CaMgSi_2O_6$–$Mg_2Si_2O_6$ is not necessarily the same as this Translated into temperatures this would represent an error of +7 °C per kbar for assemblages equilibrated at a higher pressure than that of the experimental determinations

Material of composition $Di_{20}En_{80}$ yielded a homogeneous clinopyroxene at 1,620 °C and diopside and enstatite at 1,520 °C Kushiro[4] predicted an assemblage of pigeonite plus minor diopside for the 1,520 °C run Material of composition $Di_{40}En_{60}$ yielded diopside plus enstatite at 1,450 °C and 1,480 °C and a single homogeneous clinopyroxene of composition $Di_{40}En_{60}$ at 1,550 °C The two higher temperature runs lie within the reported[4] field of diopside plus pigeonite stability but fail to verify its existence Runs on bulk composition $Di_{40}En_{60}$ at 1,620 °C using homogeneous gel and glass starting materials, and at 1,614 °C using the mixture of diopside and enstatite gels, all yielded a homogeneous clinopyroxene There was again no evidence for the coexistence of two clinopyroxenes as required by Kushiro[4] He reported[4] that he was not able consistently to generate the pigeonite plus diopside assemblage It is possible that the two clinopyroxene field is a quenching phenomenon Deliberately slow quenching of the $Di_{40}En_{60}$ gel, run at 1,620 °C failed, however, to produce two clinopyroxenes,

nor were they obtained from glass or homogeneous ge starting materials of the $Di_{40}En_{60}$ composition when run at 1,620 °C in the same equipment used[4] by Kushiro We conclude that there is no stable field of pigeonite separate from a diopside field on this join at 20 kbar

In the light of these results the temperatures on the geotherm estimated by Boyd[2] may require some revision, and the method should only be applied to olivine saturated assemblages when compared with a diopside solvus determined in the presence of excess forsterite

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Crustal structures in central southern Africa

WE announce preliminary results from the geophysical reconnaissance of Botswana carried out during 1972 and 1973 Tertiary to Recent sands and sediments in the Kalahari Basin totally obscure the solid geology over 70% of the country, but these new results give exciting first indications of the disposition of geological features in the concealed 'basement' when related to the better known geology of the surrounding territories The new data also suggest the extent of the concealed sedimentary and volcanic strata of Karroo age, and their possible relationship to the recent, if slight, tectonic instability in the heart of the continental plate This work forms part of Botswana's contribution to the International Geodynamics Project[1]

A network of 2,131 gravity stations was established over Botswana's thinly populated 575,000 km² land area, over much of which access and communication by land is difficult and slow A network of gravity base stations at 23 strategic airstrips was established using two LaCoste and Romberg gravimeters
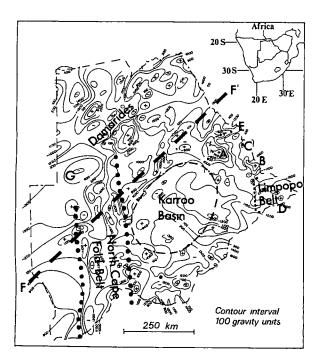
transported by light aircraft[2] and the intermediate stations were reached either by truck or helicopter Altitude control of barometric observations was provided by the 1964 primary triangulation (Directorate of Overseas Surveys) and by more recently completed precise levelling routes (Department of Surveys and Lands, Botswana) Navigation equipment on trucks was essential for position fixing Total-field magnetic observations were made at 1,300 of the gravity stations

A mean negative Bouguer anomaly of about 1,150 gravity units (1 g u $= 10^{-6}$ m s$^{-2} = 0$ 1 mgal) persists over the whole country, reflecting the isostasy of the monotonously flat continental plateau which stands at about 1,000 m above sea level, while locally Bouguer anomalies range in value from -700 g u to $-1,500$ g u

In the geologically better known areas in the east of the country the denser rocks of the isolated greenstone belts or "schist relics"[3] in the Archaean basement produce local positive gravity anomalies By far the largest of these is at Matsitama (A, Fig 1) and smaller ones are found at Tati (B), Vumba (C), Baine's Drift (D) and Maitengwe (E) Similar anomalies over schist belts in both South Africa and Tanzania have been discussed by Darracott[4,5] But even the anomaly over the Barberton Mountainland, South Africa, is much smaller, both in area and amplitude, than that at Matsitama Here causative dense rocks must extend westwards below the cover of Karroo and Kalahari sediments beyond the confines of the exposure mapped geologically and a further, arcuate, extension of the Matsitama anomaly (A–E) runs north, then north-eastwards to link up with the anomaly at Maitengwe The cause of this arcuate structure is entirely concealed by more recent cover, but a connection between the two "schist relics" was predicted on purely geological grounds[3] It is convincingly delineated by the new geophysical evidence which may therefore have considerable economic importance

The gravity 'high' in the area of the northern Transvaal and southern Rhodesia border, recognised in earlier surveys and attributed to the Limpopo Mobile Belt, quickly disappears when traced westwards into Botswana, becoming extinct west of Selebi–Pikwe This corroborates recent thinking[6] that the Limpopo Belt may terminate in this region and does not continue westwards to intersect the Damaran Belt[7] nor swing southwards to join the North Cape Fold Belt

Fig. 1 Bouguer anomaly map of Botswana showing preliminary interpretation

Contour interval 100 gravity units

250 km

In east central Botswana a large featureless area of the Bouguer anomaly map seems to characterise the Karroo Basin and suggests its western and southern limits, where there is no geological control as yet[8] An earlier study of the seismicity of Botswana[9] shows this same area as notably aseismic Together these factors tend to support Green's suggestion[8] that the deposition of the Karroo strata was controlled by epeirogenic movements of a rigid continental plate, rather than by multiple or migratory rifting, at least in the area indicated in Fig 1

The geological significance of a "Kalahari Seismicity Axis"[9], based on recent epicentre data and supported by the disposition of photogeological features, is well supported by the gravity data, where a considerable anomaly gradient follows much of the length of the axis (F–F') Seismic activity may be associated with an abrupt change in crustal thickness or density along this line This might be the edge of the foreland to the Damaran orogenic belt, an incipient Rift, or both, but whatever its significance, the Kalahari seismicity axis divides the gravity anomaly map into areas of distinctly different "texture" South-east of the seismicity axis the gravity anomaly field is largely featureless, devoid of steep gradients and localised anomalies, while to the north-west numerous large anomalies, both positive and negative, are evident Many of the anomalies are elongated in an approximately NE–SW direction, giving an overall impression of a strong north-easterly strike over the entire north-west half of Botswana This area includes the seismically active Okavango Delta where recent seismic refraction studies have shown[10] that some of the faults determining the south-east margin of the delta[9] are downthrown by as much as 300 m to the north-west and probably postdate the Karroo But faulting even on this scale can only make a relatively small contribution to the observed gravity anomalies

The only area of extensive rock outcrop in western Botswana is the Ghanzi Ridge where there is a major gravity "low" (G) Here the rocks are considered to be part of the Damara orogenic belt and, by extrapolation, we suggest that Damarides may underly the entire area north-west of the Kalahari seismicity axis Anomalous density distribution resulting from folding and intrusion could account for the pattern of gravity anomalies characterising this area This, however, contrasts with the findings of Darracott[11] that the Mozambique orogenic belt is featureless in gravity anomalies compared with the adjacent cratonic area in East Africa! We do, however, believe that it is significant that both the Okavango seismicity and the 'Kalahari Seismicity Axis' seem broadly associated with areas we now presume to be underlain by the Damaran orogenic belt

Striking north from the vicinity of Tshabong in southernmost Botswana a clear trend extends that recognised in the gravity survey of South Africa[12], characterised by the large positive and negative anomalies in the vicinity of Olifantshoek in the North Cape Fold Belt On the Bouguer anomaly map of Botswana this trend may be traced as far north as Ghanzi, where it seems to become engulfed in the north-easterly, supposed Damaran, trend A major negative anomaly in the regional magnetic contours south of Ghanzi is coincident with the northerly gravity trend We conclude that the North Cape Fold Belt (10^9 yr) recognised in the Cape Province continues in a northerly direction as far as Ghanzi where it becomes lost in the Damaran remobilisation (5×10^8 yr), the whole being concealed below the Karroo and Kalahari cover

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Geochronology of eastern Newfoundland

WHOLE-ROCK Rb–Sr isochrons from granitoid rocks intruded into the easternmost part of the Central Mobile Belt and the Avalon Platform of Newfoundland indicate that the bulk of the plutonic activity took place during Devonian and Carboniferous times Of the nine intrusions that were dated, only one, a Cambrian granite from the Avalon Platform, was significantly older

The Appalachian System in Newfoundland can be divided into three geological provinces, each characterised by a distinct geological history[1-3] the Western Platform, the Central Mobile Belt and the Avalon Platform Volcanic rocks of Early Palaeozoic age (the Central Mobile Belt) are sandwiched between two blocks of Precambrian basement and Palaeozoic platformal sedimentary rocks A more refined subdivision may also be possible, with the island divided into eight zones (designated A to H), each characterised by a distinct Ordovician and/or pre-Ordovician history of deposition and folding[4] The northern and western parts of the island have been studied in detail, and the geology of those areas has furnished some of the basic observational information used to synthesise models for the evolution of orogenic belts[5] and the origin and emplacement of ophiolite complexes[6] The central parts of the island have not been so well studied

Granitoid rocks make up a significant proportion of the total volume of rocks exposed in Newfoundland and contribute (Map 1231A, Geological Survey of Canada) about 40 per cent of the total area of granitoid rocks exposed in the Canadian Appalachians The ages of a few of the intrusions are known from stratigraphic evidence, and some K–Ar measurements have been made on others, but the timing of the magmatic activity remains obscure These K–Ar ages are mostly single determinations on single mineral phases, and must be interpreted with caution The isotopic ages obtained so far range from 330 to 450 Myr, with the bulk of them between 340 and 400 Myr These granites have been related to the Devonian Acadian Orogeny and the K–Ar ages seemed grossly in agreement with this opinion Other intrusions, however, are believed to be significantly older[7]

Here we summarise results of a geochronological study of granitoid rocks from Zones F, G and H in eastern Newfoundland Full details will be published elsewhere Nine intrusions were dated by the Rb–Sr whole-rock method (Fig 1)
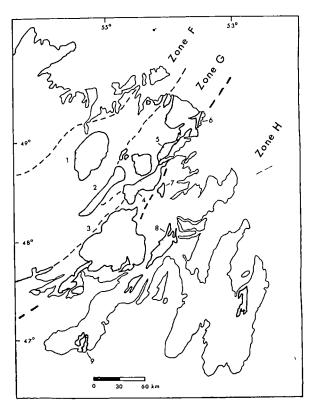
Rb–Sr ratios and concentrations were determined by X-ray fluorescence The ratios and the concentrations are considered to be accurate to $\pm 3\%$ (95% confidence level) of the quoted value Sr isotope ratios were measured on a 15-cm, 90°, solid-source mass spectrometer Our reproducibility is one part in 700 at the 99% confidence level Measurements of the Eimer and Amend Sr carbonate standard gave an $^{87}Sr/^{86}Sr$ ratio of 0 7077 $\pm$ 0 0003

The whole-rock Rb–Sr ages (Table 1) are calculated on the basis of the $1 47 \times 10^{-11} yr^{-1}$ decay constant for ^{87}Rb They indicate that (1) Of the nine granites sampled, six are either Silurian or Devonian according to the 'absolute' time scale,

and thus could fall within the generally accepted time span associated with the Acadian Orogeny (2) Bearing in mind the uncertainties assigned to each age, it seems that in Zones F and G, granite magmatism had started by the Silurian and continued into the early Carboniferous Plutonic activity on the Avalon Platform (Zone H) extended much further back, at least into Cambrian times (3) None of the granites in Zones F and G that were analysed are of pre-Middle Ordovician age This is surprising because many of the foliated leucogranites, particularly those associated with the metamorphic terrain, were considered to be that old on geological grounds[7]

Two models have been suggested for the geological evolution of eastern Newfoundland Kennedy and McGonigal[7] divided the region into three distinct terrains a basement or gneissic terrain of possibly Precambrian age, a metasedimentary terrain of pre-Middle Ordovician age, and a Middle Ordovician sedimentary and volcanic terrain Kennedy and McGonigal suggest that the gneissic rocks not only underlie the eastern part of the Central Mobile Belt but that they may also form a sialic basement to the Avalon Platform This relationship between the underlying basement and the Gander Lake Group (redefined to include only the metasedimentary terrain) resembles that of the Precambrian (in part Grenville) rocks of the Western Platform and the overlying Cambro-Ordovician sequences A similar relationship also exists between the basement gneisses of the western flank of the Central Mobile Belt and the overlying Fleur de Lys Supergroup A correlation of the newly-defined Gander Lake Group and the Fleur de Lys Supergroup is considered in terms of two sedimentary units that form continental prisms thickening from the eastern and western platforms on to the central belt

By contrast, the plate tectonic model of Strong *et al*[9] involves a south-easterly-dipping subduction zone, with the Avalon Platform forming a continental margin, and with the plutonic rocks of the Gander Lake Metamorphic Belt (Zone G) inter-

Fig 1 Sketch map (after Strong *et al*[10]) of the granitoid rocks of eastern Newfoundland The numbered granites are 1, Mt Peyton, 2, Middle Ridge, 3, Ackley City, 4, Freshwater Bay, 5, Middle Brook, 6, Cape Freels, 7, Terra Nova, 8, Swift Current, 9, St Lawrence

Table 1 The analytical data

Locality	Normative composition[10]	Petrographic notes[10]	No of points	Age (Myr)	Mean square of weighted deviates (MSWD)†
St Lawrence	Granite	Alaskite granite–rhyolite porphyry–abundant veins and fracture fillings of fluorite	4	315 ± 10*	3 2
Terra Nova	Granite	Coarse-grained granite—also microcline, megacrystic variety	4	335 ± 20	0 3
Ackley City	Granite–quartz monzonite	Orange to red, coarse-grained porphyritic biotite granite, irregular masses of alaskite and aplite	4	345 ± 10	1 8
Freshwater Bay	Granite	Megacrystic microcline granite, in parts schistose-foliation trending NE–SW Inclusions of metasediment and migmatite	4	360 ± 15	0 9
Middle Ridge	Granite	Pink medium-grained and central garnetiferous leucocratic phase	4	370 ± 10	3 8
Mt Peyton	Granite–granodiorite	Syenite, granite, diorite, monzonite	4	375 ± 15	12 7
Cape Freels	Granite	To east, homogeneous megacrystic granite—to west, augen gneiss	4	400 ± 15	0 3
Middle Brook	Granite–granodiorite	Microcline megacrystic granite—metamorphic texture in parts, augen gneisses	6	435 ± 35	3 1
Swift Current	Granite–diorite	Pink equigranular medium-grained granite, also contaminated variety with granitised volcanic and sedimentary rocks	4	510 ± 20	7 7

*95% confidence level

†This quantity gives some idea of the goodness of fit of the points to a straight line, and in the ideal case approaches a value of one if the points fit the line within experimental error[8] In practice, the MSWD is strongly dependent on the precise estimation of the analytical uncertainties, and on the total number of points used to define the isochron, so that for our results any MSWD less than about five probably represents a suitable fit of the points to a straight line Only the results from Mt Peyton show any real sign of a deviation from 'ideal' behaviour

preted as Palaeozoic intrusions above the subduction zone The metamorphic belt running along the eastern part of the Central Mobile Belt is related to the same subduction zone, and reflects metamorphic activity above a down-going plate sometime during the Palaeozoic The direction of subduction from northwest to south-east is supported by some gross geochemical trends (in particular a definite eastward increase in the average K content of the granitoid plutons of eastern Newfoundland) and by a zonation of many Newfoundland mineral deposits

To distinguish unequivocally between either of these two models by using both the geological information and the data in Table 1 is difficult, but the Rb–Sr ages provide certain important constraints Perhaps the most important finding is that most of the granites that were sampled are 400 Myr old or younger The prediction that several of the foliated granites intruded into the gneissic terrain may be of either Precambrian or pre-Middle Ordovician age is difficult to reconcile with the new data even though the higher mean square weighted deviates (MSWDs) associated with some plutons may indicate that the Rb–Sr system was disturbed sometime after intrusion The intense foliation associated with at least two of the megacrystic granites, the Cape Freels and the Freshwater Bay, suggests a much younger (post-400 Myr) period of intense deformation and/or metamorphism On the other hand, a variation in age across an ocean-arc and continent system, which would be intuitively expected from a simple model of subduction as suggested by Strong *et al*, is not at all apparent from our data, although any igneous phenomena associated with a downgoing plate are not going to be straightforward in terms of either age or spatial distribution

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Origin of gasoline range alkanes in the deep sea

IT is believed that C_4 to C_7 hydrocarbons in petroleum are formed by the cracking of organic matter at depths generally exceeding 1,000 m at temperatures in excess of 50 °C (refs 1–3) Also, none of the alkanes in the butane–heptane range are formed biologically as far as is known at present Consequently, it is thought that they do not occur in shallow, Recent sediments In 1962, I analysed 22 samples of Recent sediments from 7 different environments and verified that these hydrocarbons were not present at the p p m level[4] although traces of a few hydrocarbons such as butane, isobutane, isopentane and n-heptane have been found[5-8] No identification of individual hexanes or heptanes has been reported except when there has been clear evidence of seepage from deeper source sediments[9]

Using more sensitive techniques I have detected the widespread occurrence of practically the entire suite of C_4–C_7 alkanes of petroleum at the ng g^{-1} level in shallow, low temperature marine sediments The yields generally correlate with organic carbon This suggests that diagenetic reactions which form traces of C_4–C_7 hydrocarbons are occurring much earlier and at much lower temperatures than most geochemists had expected

The analytical technique[10] consists of placing frozen core material in a metal container completely filled with water and then introducing a known volume of helium to form a gas cap After thawing, the container is placed in a boiling water bath, then agitated and a sample of gas is withdrawn and analysed on a gas chromatograph fitted with a 200 foot, 0 01 inch internal diameter hexadecene-hexadecane-Kel F capillary column Each peak was identified against a known standard.

Table 1 Total yield of C_1–C_7 alkanes from Deep Sea Drilling Project (DSDP)

Location*	DSDP Core no *	Interval (cm)	Age	Sediment depth (m)	Lithology	Organic carbon (weight %)	C_4–C_7 alkanes (parts per 10^9)†
1. Gulf of Mexico 23°N 92°W	1-3D-1-2	70–150	Upper Pleistocene	28	Silty clay	0 81	18
2 Off California 39°N 127°W	5-34-4-4	86–126	Lower Pliocene	113	Clay mud	0 56	5 5
3. Off California 39°N 127°W	5-34-5-4	0–40	Lower Pliocene	120	Clay mud	0 47	28
4 Bengal Basin 9°N 91°E	22-217-16-4	10–30	Palaeocene	416	Nannofossil chalk	0 04	0 47
5 Bengal Basin 9°N 86°E	22-218-27-2	0–10	Middle Miocene	769	Clayey silt	0 14	6 1
6. Gulf of Aden 14°N 52°E	24-233A-7-4	0–20	Lower Pliocene	229	Nanno-ooze	2 95	109
7 Indian Ocean 33°S 39°E	26-250A-10-6	6–26	Lower Pliocene	414	Silty clay	0 20	0 46
8. Indian Ocean 31°S 88°E	26-254-25-6	120–140	Miocene	228	Clay mudstone	0 10	0 88
9. South of Tasmania 49°S 147°E	29-280A-6-2	125–150	Upper Oligocene	109	Diatom silt	0 36	18
10. South of Tasmania 49°S 147°E	29-280A-19-0	0–50	Lower Eocene	443	Silty clay	0 83	1,284
11. South of Tasmania 49°S 147°E	29-280A-21-1	130–150	Lower Eocene	511	Silty clay	0 61	6,386
12 West of Tasmania 42°S 143°E	29-282-4-0	33–55	Upper Miocene	28	Nanno-ooze, clay	0 21	32
13 West of Tasmania 42°S 143°E	29-282-18-1	90–100	Lower Eocene	295	Clayey silt	4 8	1,964
14 Sea of Japan 39°N 138°E	31-299-22-1	0–110	Pleistocene–Pliocene	200	Clay	0 95	22

* Exact well locations and core descriptions are published in the Leg Reports of the DSDP, US Government Printing Office, Washington, DC DSDP core numbers indicate in order leg-hole-core-section

† Parts per 10^9 by weight, or ng per gram of dry sediment

About 5% of the samples analysed so far, mostly from abyssal plain areas, have shown no detectable hydrocarbons even though the method is sensitive to 5 parts per 10^{12} in a 100 g sample This indicates there is no continuous source of contamination at the levels studied

Table 1 shows the yield of C_4–C_7 alkanes from 14 deep sea samples The samples range in age from Pleistocene to Palaeocene and in sediment depth from 28 to 769 m Organic carbon contents vary from 0 04 to 4 8% Nannofossil chalk (Table 1, no 4) had the lowest yield, and oozes and clays from the outer continental margins (Table 1, nos 1–3, 6, 9–13) had the highest yields The yield of C_4–C_7 alkanes generally increased with increasing organic carbon content except in the vicinity of Tasmania where very high yields in the range of 1,000–6,000 parts per 10^9 were obtained These high yields are associated with a basalt intrusion which occurs at a depth of about 520 m in Hole 280A Previous studies[11,12] have shown that the high temperatures caused by igneous intrusions can generate large quantities of hydrocarbons from the organic matter in adjacent shales Distribution of these hydrocarbons can be erratic because of variations in migration pathways, but yields are invariably higher than average The three deepest samples near Tasmania have a mean yield of 3,211 μg alkanes per gram of carbon, which compare with a mean yield of 22 μg alkanes per gram of carbon for the rest of the samples This suggests that some of the alkanes in the deep Tasmanian samples have migrated from sediments heated by the intrusion

When factors such as temperature are relatively constant, a correlation between hydrocarbon yields and the total organic carbon content of the sediment indicates that the alkanes are autochthonous although there is no way to prove conclusively that this is so When the yield of C_4–C_7 alkanes is plotted against organic carbon, excluding the three deep Tasmanian samples, the correlation coefficient is +0 75 This indicates that those alkanes are autochthonous There is apparently no correlation with lithology, age or depth The deepest sample in any one hole generally has the highest yield Marked increases in yield, however, occur over very short distances, as in the first two samples of Hole 34 These differences cannot be a

result of the temperature increase with depth The highest temperature to which these samples have been exposed is estimated to be about 45 °C for the sample from the deepest part of the Bengal Basin Most samples have never been above 25 °C More likely, differences in yield, as in Hole 34, are related to variability in the type of organic matter A few samples were treated with HF and HCl to remove the mineral matter, and the organic residues were examined microscopically Organic matter in the deep sea samples was generally amorphous whereas the clays from basins along continental margins contained woody fragments, spores and pollen The Indian Ocean sample from Hole 254, which is over 1,500 km from any continent, showed a surprising amount of woody material. Palynological studies[13] showed, however, that these sediments were deposited in a shallow lagoon during Miocene times

Individual hydrocarbon yields for eight representative samples are shown in Table 2 Results to date indicate that certain hydrocarbons form very early during diagenesis, whereas others form very late The earliest formed hydrocarbons are generally the butanes, pentanes, 2- and 3-methylpentanes and methylhexanes, *n*-hexane, *n*-heptane, and methylcyclohexane The hydrocarbons that seem to form latest are 2,2-, 2,3- and 2,4-dimethylpentanes, 1,*cis*-3 and 1-*trans*-2-dimethylcyclopentanes, and 3-ethylpentane About 1 sample in 20 contains a trace of 2,2,3-trimethylbutane A few samples contain olefins which are now being examined using mass spectrometry Large anomalies in individual hydrocarbon yields are scattered erratically through the samples For example, *n*-pentane constitutes 43% of sample 7 and 42% of sample 6 Sample 13 contains 31% 2-methylhexane When it is realised that an individual hydrocarbon represents only 0 0001% of the organic matter, it is understandable that such anomalies can be attributed to a very small part of the organic matrix For example, sample 13 may contain a few easily reducible compounds with 2-methylhexane chains from the original plant material It can be expected that such anomalies would disappear when the organic matter was buried to sufficient depth to generate large quantities of a variety of hydrocarbons by thermocatalytic reactions

Table 2 Individual C_4–C_7 alkane yields (ng per gram of sediment)

	7*	8	9	1	14	12	6	13
Isobutane	0.082	0.033	0.34	0.25	1.82	0.29	2.5	8.7
n-Butane	0.021	0.040	1.63	1.36	2.61	1.49	1.2	25.6
Isopentane	Abs	0.31	0.54	0.54	0.21	0.68	1.3	32.5
n-Pentane	0.20	0.21	4.64	6.55	0.42	4.41	45.8	93.6
2, 2-Dimethylbutane	Abs	Abs	0.65	0.17	0.64	0.60	0.5	12.4
Cyclopentane	Abs	Abs	0.30	0.17	0.29	0.45	3.3	185
2, 3-Dimethylbutane	0.092	0.12	0.23	Abs	0.17	0.41	Abs	30.0
2-Methylpentane	Abs	Abs	0.59	2.54	1.74	1.19	3.0	43.3
3-Methylpentane	0.026	Abs	3.17	0.71	0.61	0.98	1.8	21.2
n-Hexane	0.009	0.07	0.68	1.33	1.24	1.24	6.9	36.5
Methylcyclopentane	Abs	0.04	0.38	0.50	0.64	2.36	2.6	18.1
2, 2-Dimethylpentane	Abs	Abs	Abs	Abs	Abs	Abs	Abs	28.4
2, 4-Dimethylpentane	Abs	Abs	Abs	Abs	0.56	Abs	Abs	14.1
Cyclohexane	Abs	Abs	0.03	0.30	0.99	1.18	3.0	35.3
3, 3-Dimethylpentane	Abs	Abs	Abs	0.12	0.78	0.56	0.6	9.3
1, 1-Dimethylcyclopentane	Abs	Abs	Abs	0.12	0.76	0.42	0.6	35.3
2-Methylhexane	0.02	Abs	0.65	0.4	1.56	1.39	3.6	618
2, 3-Dimethylpentane	Abs	Abs	Abs	0.16	Abs	1.22	Abs	Abs
1, cis-3-Dimethylcyclopentane	Abs	Abs	Abs	0.22	Abs	Abs	Abs	62.4
3-Methylhexane	Abs	0.05	0.86	0.18	1.30	2.01	5.7	35.7
1 trans-3-Dimethylcyclopentane	Abs	Abs	Abs	0.66	0.31	2.80	Abs	63.0
1 trans-2-Dimethylcyclopentane	Abs	Abs	Abs	Abs	0.78	Abs	4.8	118
3-Ethylpentane	Abs	Abs	Abs	Abs	0.67	Abs	Abs	12.1
n-Heptane	0.005	0.005	1.40	0.62	1.09	2.53	9.8	58.8
1, cis-2-Dimethylcyclopentane	Abs	Abs	Abs	0.22	0.39	1.56	1.1	34.5
Methylcyclohexane	Abs	Abs	1.61	0.84	2.14	4.63	10.5	332
Total	0.46	0.88	18	18	22	32	109	1,964

* Sample numbers as in Table 1.
Abs = Absent

Hydrocarbon ratios even at this early stage of generation are similar to those in crude oil for some, but not all, hydrocarbon pairs. For example, the ratio of 2- to 3-methylpentane in crude oil averages 1.7 (ref. 14). The mean ratio in the samples of Table 2 is 1.9. The ratio of 1-trans-2- to 1-cis-2-dimethylcyclopentane is about 2.7 in crude oils and 3.3 in these samples. Between methylcyclohexane and cyclohexane the ratio is 2.5 in crude oil and 4.4 in these samples, excluding sample 9 which has almost no cyclohexane. An inverse relationship is noted in the ratio between 2 and 3-methylhexane; it is 0.8 in crude oils and 1.1 in these samples, excluding the anomalous sample 13.

All studies to date indicate in general that some hydrocarbons form earlier than others. When yields at specific locations are compared, however, it seems that some organic matter forms a wider spectrum of hydrocarbons more readily than others. Thus, the Pleistocene sample from a depth of 28 m in the Gulf of Mexico contains 5 more hydrocarbons in the C_4–C_7 range than the Oligocene sample from a depth of 109 m south of Tasmania, even though both samples have a total of 18 ng of hydrocarbon per gram of sediment.

The occurrence of these gasoline range hydrocarbons in shallow marine sediments may be an early indicator of the capability of sediments to generate petroleum. On the other hand, a significant point of this study is that time and the particular nature of the organic matter are not alone sufficient to ensure the generation of commercial quantities of petroleum hydrocarbons. A higher temperature is required than the 15–45 °C range recorded here. For example, the Eocene clays associated with the Lake Maracaibo oilfields in Venezuela have about 1 mg of C_4–C_7 alkanes per gram of carbon at about 75 °C compared with the 18 µg per gram of carbon in the shallow Eocene samples of this study[4]. In the Jurassic sediments of the Paris Basin, there is[3] about a 100-fold increase in the C_4–C_7 alkanes in going from sediments at about 35 °C to those at about 100 °C.

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Acoustic observations of suspended particulate matter in the ocean

CLOUDS of suspended particulate matter in the ocean have been observed by satellite[1], sensed by nephelometers[2], and sampled by water bottles. We present here evidence that it may be possible to observe oceanic suspended particulate matter acoustically. The particulate matter discussed is thought to have arisen from a dredging operation. It is also thought that the suspended matter is present at lower concentrations than any in any other case recorded acoustically[3]. Certain questions, as yet unresolved, regarding acoustic scattering strengths are also discussed.

An experiment to determine the feasibility of acoustic surveys of suspended sediments was conceived during field testing of a 20-kHz LODAR echo-sounding system. The LODAR system has a downward-looking acoustic beam of 12° by 18° and a pulse duration of 2.2 ms. Initial acoustic records of a cloud-like feature were coincident with visual sightings of suspended sediment down-current of a working dredge. During this initial experiment, the dredge was operating for five days out of eight and data were taken on both incoming and outgoing tides. In conjunction with the LODAR records, measurements were made with expendable bathythermographs (XBTs) and a continuous recording light beam transmissometer (LBT).
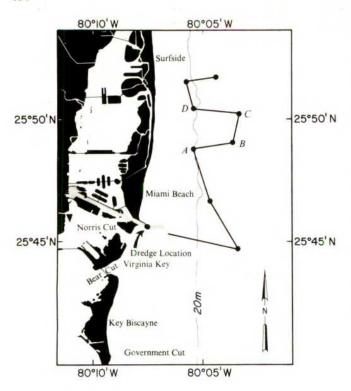
Fig. 1 Typical trackline in area of investigation (May 22, 1974, RV Virginia Key). A–D indicates locations shown on Fig. 2.

On the five days when the hydraulic suction dredge was working, a visible cloud of suspended sediment and the acoustic 'cloud' were observed down-current of the dredge. On incoming tides the sediment flowed into the bay where the water is too shallow to operate the LODAR system. On outgoing tides the suspended sediment flowed out of the channel; the surface manifestation disappeared between 200 and 600 m seaward of the channel, depending on the state of the sea. Figure 1 is a typical trackline and Fig. 2 is the acoustic record from part of that track. The same general trackline was traversed each day. The minimum volume of the cloud estimated from acoustic records was about 60,000 m³. During the three days when the dredge was not in operation, clouds were not observed either by the acoustic equipment or by visual observation of any surface manifestation.

The continuous recording LBT records the percentage transmission of a beam of white light 2 cm in diameter over a 1-m path; filters maximise the sensitivity at 475 nm. Although the light transmission characteristics involve more than one parameter[3,4], the instrument is useful for obtaining approximate particulate concentrations and distributions. LBT station depths were limited by the length of telemetering cable aboard,

but shallow casts (less than 100 m) indicated a reduction in transmittance of between 10% and 15% in the upper regions of the cloud. The developed cloud occupied a region between inflection points on the XBT temperature traces (Fig. 3). A volumetric concentration on the order of 0.01–1%, obtained from the acoustic data, enabled us to carry out a first-cut check of acoustic and transmissometer readings. For the LBT used[5], a reduction in transmittance from 85% to 60% implies a particulate concentration of roughly 0.03%, so that the acoustic and transmissometer devices are consistent.

The observed acoustic 'cloud' cannot be accounted for either by temperature anomalies or by a package of contaminated bay water which would occur independently of dredging operations (see ref. 6). Temperature anomalies, however, seem to influence the location in depth of the acoustic cloud which occupies a region between inflection points on the XBT records (Fig. 3). This observation is consistent with the finding that temperature-induced density gradients are an important controlling factor in the transport of fine-grained sediment[7]. Large biological reflectors cannot account for the cloud as there is no evidence of point source reflectors on the records. The possible role of small biota in producing such a cloud requires further investigation. Although unlikely, the stirring up of bottom nutrients by the dredging operation may have produced a short-lived bloom of microscopic biota.

It is unlikely that the cloud results from microbubbles produced by organic processes in the sediment and released by the dredging operation. An extraordinary amount of decay of organic matter would have to occur to produce clouds of the magnitude observed on every outgoing tide when the dredge was in operation. Such extraordinary quantities would have been observed as seeps emanating from the bottom when the dredge was inoperative.

The observed behaviour and properties of the acoustic cloud support our interpretation that it is the product of acoustic scattering from suspended sediment particles in the water column. The nature of a suspended sediment cloud and its movements would be affected by the tides, temperature-induced density gradients, and the northward flow of the Gulf Stream current. The observed cloud varied in size, shape, and location corresponding to the changes in dredging operations and tides. The tides affect both the direction of flow in Government Cut and the flow of warmer bay waters out to sea on outgoing tides, thus inducing the density gradients at the outflow of the cut. Also, the observed acoustic clouds moved to the north on leaving the cut, with the easternmost extent of the cloud approaching the Gulf Stream.

How is it, though, that particles with diameters that may be of the order of 1–100 μm can reflect detectable sound signals at 20 kHz? The present hypothesis is that the sediment cloud may have regions of relatively high particulate concentration which are detectable but not resolvable using the acoustic system described here. Studies are under way to determine the validity of that hypothesis.

Theoretical work and further field work are under way to
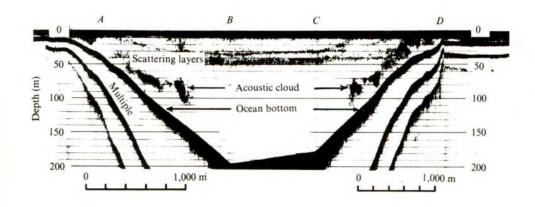
Fig. 2 Acoustic LODAR record along typical trackline, locations as indicated on Fig. 1. The shallow scattering layer is indicated. May 22, 1974.

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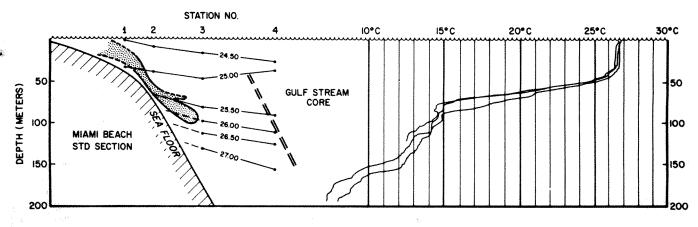
Fig. 3 Sketch of the acoustic cloud with overlay of Sigma-T (σ_T) curves (T. Lee and D. Mayer of National Oceanic and Atmospheric Administration) with XBTs on same scale, May 22, 1974. $\sigma_T = (\rho - 1) \times 1,000$ where ρ = water density). σ_T is obtainable from STD (salinity-temperature-depth) measurements. Gulf Stream core based on salinity of March 27, 1974.

develop what may prove to be a valuable survey tool, with immediate applications for the sedimentologist, geologist, ecologist and pollution monitoring agencies.

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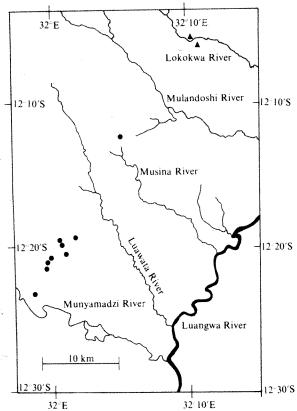
Vertebrate localities in the Karroo System of the Luangwa Valley, Zambia

PROLIFIC vertebrate localities within the Karroo System of the northern part of the Luangwa Valley in Zambia have been known since Dixey's investigations of 1928 and 1935[1]. They were the subject of extensive collecting in 1960–61 by the then Geological Survey of Northern Rhodesia[2], and again in 1963 by a joint British Museum (Natural History)–University of London expedition[3]. Two fossiliferous horizons are recognised in the Luangwa Valley, the Madumabisa Mudstones corresponding to the Upper Permian *Daptocephalus*-zone of South Africa, and the Ntawere Formation which is regarded as a little older than the Manda Formation of Tanzania and corresponds approximately to the Beaufort–Stormberg boundary of the Karroo System. In 1972 members of the Geological Survey of Zambia investigated the middle Luangwa Valley in and around the game reserves, discovering two new, potentially important localities. The first lies in the Munyamadzi Corridor between the northern and southern game reserves and is an exposure of the Madumabisa Mudstones yielding abundant therapsid and some pareiasaur remains. The fossils

are, however, preserved in calcareous mudstone nodules which lack the extensive, hard ferruginous covering which makes the specimens from the Upper Luangwa so difficult to prepare. The second is an exposure of the Ntawere Formation in the northern game reserve and yielded archosaur-like teeth along with the bivalve *Unio karrooensis*. As a consequence of these discoveries members of the Oxford University Museum were invited to participate in an expedition during the summer of 1974, to collect fossil vertebrates from the series of new localities in the middle Luangwa Valley.

North of the Munyamadzi River extends a nearly flat alluvial plain, beyond which the terrain rises about 70 m in 6–7 km. This hilly belt is dissected extensively by stream beds and eight localities were found within the 'donga' type erosion system associated with these streams. Most of the localities lay just below a remnant of horizontal grit or sandstone on an interfluve and consisted of small gullies cutting through the soil and soft rock, which often coalesce to form wide, shallow, bare hollows. The fossiliferous rock is a near-horizontal, brown and grey, fine-grained calcareous mudstone, with frequent nodules that are of the same rock type but are more calcareous, and in which the fossils themselves occur. Large amounts of bone have weathered out and lie strewn over the surface but numerous good specimens were found still *in situ*.

A basically similar locality was found on the Musina River some 18 km further north, apparently in the same geological horizon. The specimens collected from these Madumabisa Mudstones were characteristic of the *Daptocephalus*-zone of South Africa. They consisted of abundant dicynodont skulls often with postcranial bones associated, about a dozen gorgonopsid skulls including one with much of the postcranial skeleton, two whaitsiid-like therocephalians, a complete procynosuchid skeleton resembling *Parathrinaxodon proops*[4], a pareiasaur skull and partial postcranial skeleton and, at one particular locality, numerous remains of fishes, including hybodont-like fin spines, palaeoniscid scales and the partial skull of an *Acrolepis*-like palaeoniscid. All these specimens have since proved very suitable for the acetic acid method of preparation.

Two localities along the Lokokwa River in the northern game reserve were also investigated. They seem to be exposures of the Ntawere Formation and consist of a sequence of mottled red-brown and greenish mudstones and siltstones with some thin, light grey, flaggy sandstones and lenses of intraformational conglomerate of calcareous pellets. Vertebrates were, however, extremely scarce and the only specimen of likely value is a piece of the conglomerate containing part of a small archosaur skeleton. Otherwise only a few surface fragments of labyrinthodont and dicynodont nature, and a large archosaur tooth were found, along with abundant *Unio karrooensis* specimens.

Fig. 1 Karroo localities of the middle Luangwa Valley. ●, Madumabisa Mudstones; ▲, Ntawere Formation.

The collection is now in the Oxford University Museum where it is under preparation. I express my gratitude to the staff of the Geological Survey of Zambia, particularly Dr Alan Drysdall for the initial invitation and Mr and Mrs Colin Kerr, for their cooperation throughout the expedition. I also thank the Wildlife Department and the Monuments Commission of Zambia for the necessary permits, and the Royal Society for a grant. Mr H. P. Powell of the Oxford University Museum and Mr C. D. Kerr of the Geological Survey of Zambia accompanied me during this expedition.

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Do children draw men with arms coming out of the head?

CHILDREN'S drawings raise formidable taxonomic problems. Consider the two extremely common drawings in Fig. 1. There are good reasons for selecting the 'conventional man' as a standard against which to assess the 'tadpole man' but we do not know how to do it. It may be that the tadpole man is lacking a trunk[1-4] or that it has the trunk amalgamated with the head[4,5] (and at least two other descriptions are also plausible). In practice, authors have tended to opt for the following subset of possible hypotheses: (1) the trunk is omitted and the arms attached according to a rule 'attach arms to head'; (2) the trunk is omitted and the arms drawn on the head by default even though the child has a rule specifying

the trunk as a locus of insertion for limbs; (3) the trunk is not differentiated from the head, and therefore the rule 'attach arms to trunk' is not violated. The present experiment was designed to test the plausibility of such accounts. Children were given incomplete figures and asked to draw limbs. The results show that the tendency to attach arms to the head or the trunk can be brought under lawful control in such a way as to discriminate against the above hypotheses in favour of one based on the concept of a 'production error'.

As in any binary-choice experiment, the primary goal is to find a set of stimuli which will reliably affect the relative strengths of the two responses under examination, namely, attaching arms to the head or to the trunk, at the same time as satisfying the condition that the stimuli occur at approximately equal intervals on an underlying scale. Such a set is series *a* in Fig. 2, in which each stimulus pair sums to the same overall height, with the central pair being of equal size to give a point of objective and subjective equality (as established in a pilot study). From a classical psychophysical standpoint, series *b* is experimentally somewhat neater since it is based on the method of constant stimuli, in which a fixed 'standard' is paired with varying 'comparison' stimuli. In the present case, one of each pair of circles is held constant, at a diameter of 3 cm, with the other circle varying on an equal interval scale, although this allows for covariance in the overall height. Both series were used in the present experiment. Similarity of the results obtained from the two series will therefore produce confidence in the assumptions involved in the scaling procedures adopted.

Children (140) in local playgroups aged between 2, 3, and 4 yr (mean = 3.8, s.d. = 0.7) were each asked to draw a man on A4 paper. They were then individually asked to complete a drawing consisting of a head. Then each was presented with a booklet containing a random ordering of one of the series shown in Fig. 2 and asked to complete each drawing before turning the page to the next. The locus of insertion of the arms was noted, and the results conditionalised on the spontaneous drawings by dividing subjects up into groups according to whether they spontaneously drew conventional men, tadpole men, or scribbles.

Fifty-four subjects drew a conventional man (group M), 33 drew a tadpole man (group T) and 35 produced scribbles (group S). The remainder, who largely produced disjointed conventional men, were ignored for present purposes. Analysis of variance showed that group M were reliably older than groups T and S (mean = 4.0, 3.7, and 3.4 yr, respectively) at the 5% level of significance. Groups M and T tended to be consistent when given the predrawn head in that 48 out of 54 of group M turned it into a conventional man and 28 out of 33 of group T turned it into a tadpole. Interestingly, none of group S scribbled, and 20 of them produced a tadpole man, whereas 15 produced a conventional man. When given the series of incomplete drawings, every child put the legs on the trunk. The results for arm attachment is shown in Table 1. Clearly group M remained relatively consistent in attaching arms to the trunk. The results for groups T and S seem to resemble one another. Calculation of Cochran's *Q* confirms

Fig. 1 Drawings of a man (*a*) conventionally, (*b*) as a tadpole figure, by two preschool children.

the unequal distribution of locus of attachment across the conditions, and linear contrast on proportions[6] is significant for group T (95% confidence limits being 3.68–1.68 for series *a*, 3.56–0.36 for series *b*). For group S, the linear trend just fails to reach acceptable significance (95% confidence limits being 2.00 to −0.12 for series *a*, 3.64 to −0.28 for series *b*). Thus there is clear evidence that for the individuals in group T, the larger the head in relation to the trunk the greater tendency to draw arms on the head. Thus group T can attach arms to the trunk but equally can be led to ignore the trunk. The same may well be true of group S, but the evidence is equivocal.

The results for group T are evidence against the operation of the stable rules 'attach arms to head' or 'attach arms to trunk' which hypotheses (1) and (2) promote. With regard to hypothesis (3), the results show that a trunk-attachment rule may be violated. In other words, any putative rule operates only in interaction with the particular graphic configuration. Therefore, rather than regarding the drawing as a direct expression of the child's conceptual knowledge[7], we should consider what kind of performance factor could result in the

Table 1 Proportion of each group of subjects who attached arms to the head rather than the trunk of an incomplete figure, as a function of head–trunk ratio

		Group M (n = 27)	Group T (n = 17)	Group S (n = 13)
Series *a*				
Head–trunk	0.1	0.00	0.06	0.15
ratios	0.7	0.00	0.06	0.15
	1.0	0.00	0.18	0.15
	1.5	0.07	0.41	0.31
	8.5	0.11	0.76	0.54
		Group M (n = 27)	Group T (n = 15)	Group S (n = 16)
Series *b*				
Head–trunk	0.3	0.00	0.00	0.12
ratios	0.6	0.04	0.20	0.12
	1.0	0.07	0.27	0.06
	1.7	0.07	0.53	0.44
	3.8	0.19	0.73	0.81

observed lawful variation. We suggest that a useful concept is 'production error', namely a characteristic defect in an executive routine, according to the following argument.

The child has the task of constructing a complex spatial array by bringing into relationship graphical elements which can only be produced in serial order. Typically children and adults talk about, and draw, a man in rather a fixed sequence: head, trunk, arms, legs. Published surveys on drawings[8-11] suggest that if a drawing is incomplete, it tends to be the trunk and arms which are partially drawn or omitted. Note that the trunk is the second element in the pair head–trunk. With regard to the limbs, it was observed in the present experiment when group T produced a spontaneous tadpole man that 80% of the sample drew the limbs in the order legs–arms ($\chi^2 = 5.12$, 1 d.f., $P < 0.05$), that is, the opposite way round to conventional description. Thus for this group of subjects, we propose that their production sequence would typically be head–trunk, legs–arms, and that irregularities are prone to occur in the second element of each pair, that is, the children are liable to sample from the first element in each category, resulting initially in a head plus legs. This means that the child produces a centralised drawing in which the pencil is brought back to a central configuration rather than continuing on a vertical path. (It is this process that the incomplete drawings elicit.) This analysis accords both with Kellogg's[12] classification of drawings (though she overinterprets centralisation as radial symmetry) and with recent evidence[13] showing that for young children, local configurational constraints may preclude them from effectively planning vertical orientation in human figure drawing. The reason why legs are the preferred limbs may be attributable to an early bio-mechanical bias towards vertical strokes, revealed in free scribbling[14], since in simple drawings
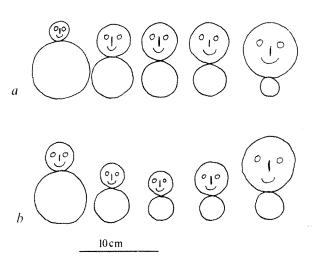
Fig. 2 Freehand incomplete drawings presented for completion. Series *a* constructed by holding overall size constant, series *b* by varying size around a constant component.

such "postural propensities are powerful enough to assert themselves"[15].

Thus we suggest that the tadpole man is associated with production problems in programming spatial layout rather than with any peculiar conceptual scheme. Note that group S, the scribblers, were also prone to head-attachment of the arms. In following up some individual scribblers we have found that they are prone to this variability for some months before they produce a recognisable conventional man or tadpole (in one case for over a year). This suggests that such drawing phenomena[16] may be indices of general production problems, rather than being simply specific solutions which arise through repeated 'near misses' in drawing a man. The general problems may well be ones of serial-order programming. In this case, the common practice of scoring a single human-figure drawing may be unsuitable as a method of assessing conceptual intelligence in children of the age range studied here, in that the face validity of the draw-a-man test is challengeable and the completion task permits abilities to be shown by scribblers which they cannot show simply on the demand "draw a man".

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Discrimination between parasitised and unparasitised hosts in the parasitic wasp *Pseudeucoila bochei*: a matter of learning

THE female of the parasitic wasp *Pseudeucoila bochei* (Hymenoptera : Cynipidae) lays her eggs in larvae of different *Drosophila* species, and only one parasite emerges from each host. The parasite is able to discriminate between parasitised

and unparasitised host larvae[1], but this ability does not completely prevent super-parasitisation. On investigating the probable causes of super-parasitisation, we discovered that the female wasp has to learn to discriminate, learning being that process which manifests itself by adaptive changes in individual behaviour as a result of experience[2].

Six possible causes were analysed, two of which proved to play an important role in the explanation of super-parasitisation (see Table 1). One of these—which must be related to the

Table 1 Possible causes of super-parasitisation by *Pseudeucoila*

Cause	Importance
1. A female lays more than one egg at an oviposition	—
2. A female does not recognise hosts parasitised by another female	—
3. A female lays a second egg after the first oviposition within the period which is needed for building up the factor which causes avoidance of super-parasitisation	±
4. Two or more females lay eggs simultaneously in one host	±
5. A female's tendency to oviposit increases when she encounters only parasitised hosts for a long period; she will lay eggs in these	+
6. A female has not yet learnt to discriminate	+

—, Never found.
±, Not important.
+, Important.

variation in the tendency of the wasp to oviposit—will be discussed in more detail elsewhere. Here, we report that in certain conditions this tendency may be strong and thus cause a high degree of super-parasitisation. This may happen when the parasite–host ratio is very high and/or when the time during which the hosts are exposed to the parasite(s) is very long.

The other cause, that the wasp has not yet learnt to discriminate, would very easily lead to a high degree of super-parasitisation. During experiments, parasitised larvae were offered to parasites which had never laid eggs before. The inexperienced parasites accepted these unsuitable host larvae (Table 2) as easily as unparasitised hosts (Table 3). The total number of contacts with, and rejections of, the parasitised larvae (Table 2) was tested against the total number of contacts with, and rejections of, the unparasitised larvae (Table 3): $\chi^2 = 1.4, P > 0.05$. Note that the general frequency of contacts was lower in the tests with parasitised larvae. The explanation of this phenomenon will be published elsewhere.

Inexperienced wasps may parasitise many already parasitised hosts in succession and thus waste a large number of their eggs. The parasites did refuse some of the larvae, although this also occurred in the control experiment, in which only unparasitised hosts were presented (Table 3).

If a batch of unparasitised larvae is presented first, and a batch of parasitised hosts thereafter, the wasps will almost completely avoid ovipositing in the latter (Table 4:

Table 2 Number of contacts with and rejections of hosts by inexperienced wasps during three or five tests of 30 min

Female	No. of tests	Total no. of contacts	Total no. of rejections
1	3	43	1
2	3	33	6
3	5	75	11
4	5	58	9
5	5	66	0
Total	21	275	27

Host larvae containing one parasite egg were offered in each test. The average number of contacts and rejections per test was about the same. After an oviposition the host was removed and replaced by a new, once-parasitised host.

Table 3 Number of contacts with and rejections of hosts by inexperienced wasps during five tests of 30 min

Female	No. of tests	Total no. of contacts	Total no. of rejections
1	5	98	5
2	5	72	4
3	5	142	6
4	5	95	21
5	5	110	1
Total	25	517	37

Unparasitised host larvae were offered in each test. The average number of contacts and rejections per test was about the same. After an oviposition the host was removed and replaced by a new, unparasitised host.

$\chi^2 = 47.3, P < 0.005$). The age of the wasps has no influence on their ability to discriminate; the only factor that matters is whether or not they had oviposited in an unparasitised host. From then on the parasites are able to distinguish parasitised from unparasitised hosts. These experiments show that the wasps have to learn to discriminate between parasitised and unparasitised larvae.

We discovered another phenomenon, which is probably related to the former. The inexperienced wasps may learn to discriminate, even when they encounter none but parasitised larvae containing for example one or two eggs. In this case they distinguish between hosts with one egg and hosts with two eggs, and avoid oviposition in the latter. As soon as the wasps encounter mainly hosts containing two eggs they leave the site.

We have shown previously[1] that the wasps not only discriminated between parasitised and unparasitised hosts, but also distinguished hosts with different numbers of eggs. At that time the functional significance of this ability remained rather obscure. In view of our present findings, however, learning may

Table 4 Number of contacts with and rejections of hosts by inexperienced wasps during 30 min of observation

Female	Test no. 1 Unparasitised		Test no. 2 Parasitised	
	No. of contacts	No. of rejections	No. of contacts	No. of rejections
1	8	0	38	36
2	23	1	34	34
3	16	0	31	30
4	14	0	24	24
Total	61	1	127	124

Unparasitised host larvae and once-parasitised host larvae were offered in succession. After an oviposition the host was removed and was replaced by an unparasitised host (test number 1) or a parasitised host (test number 2).

be seen as an important adaptation: if inexperienced wasps arrive at sites in which only parasitised larvae containing one egg are present, they do not continue laying (and thus waste all their eggs!), but leave the site when they have gradually encountered more and more larvae with two eggs. If, as we assume, at each oviposition some 'marking' substance is injected into the host (or is produced by the host), the wasp seems to be able to distinguish different concentrations of this substance, and not just notice its presence or absence.

We have presented[1] some mathematical models on the distribution of parasite eggs over the hosts. These models were based on a large number of egg distributions found in experiments. The majority of these experiments, however, were made with inexperienced wasps. We found substantial super-parasitisation in quite a number of cases, one of the causes of which may be that the inexperienced wasps used in the experiments did not start to parasitise at the same time. Since in each vial more than one wasp was present, wasps that started to parasitise later would have a better chance of meeting, as their first hosts, already parasitised larvae. These larvae

would most probably have been parasitised again by these inexperienced females.

Clearly, the larger the number of inexperienced wasps present in each vial, and the larger the differences in starting time of parasitisation between them, the higher the degree of super-parasitisation will be.

At that time we assumed that super-parasitisation was caused mainly by a strong tendency to lay eggs. In view of these new findings it seems that this would certainly apply to experienced wasps but that most of the super-parasitisation by inexperienced females should be attributed to the fact that they had not yet learnt to discriminate.

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Evolution and distribution of left-handed and planispiral coiling in snails

MOST extant snails (Gastropoda) are characterised by dextral (right-handed) coiling of the shell. Sinistral (left-handed) and planispiral (bilaterally symmetrical) coiling are uncommon among gastropods, particularly in the sea[1]. The preponderance of one type of asymmetry over another contrasts with the approximately equal numbers of right-handed and left-handed stocks of conispirally coiled fossil nautiloid and ammonoid cephalopods[2,3], and the roughly equal occurrence of inequivalve bivalves in which either the left or the right valve is larger[4].

There is no evidence that right-handedness is functionally superior or inferior to left-handedness. Here I attempt to explain previously unrecognised anomalies in the ecological and geological distribution of left-handed and planispiral coiling in gastropods by specifying some conditions of reduced stabilising selection under which the mechanisms giving rise to these coiling types could operate. My discussion is restricted to shells of adult snails. Limpet-like forms with bilateral symmetry as well as exceptional instances of sinistral coiling in otherwise dextral species are not considered.

Planispiral coiling is the presumed primitive condition in gastropods[5,6], but it has also been derived secondarily from dextral or sinistral forms in at least nine stocks of archaeogastropods (all pre-Tertiary marine), five or six stocks of freshwater and marine mesogastropods, one line of terrestrial Neritogastropoda, one line each of marine Thecosomata and Entomotaeniata, more than two lines of freshwater Basommatophora and eight planispiral stocks of terrestrial stylommatophorans (Table 1). No planispiral neogastropod is known.

Large size (2–10 cm in diameter) among planispiral shells is attained only among certain Recent land and freshwater forms (Corillidae, Systrophidae, Ariophantidae, Planorbidae, Pilidae) and among pre-Tertiary marine gastropods. All Recent marine gastropods with planispiral shells are less than 10 mm in diameter.

Topographically sinistral coiling may arise in any of three ways: First from a primitively planispiral ancestor; second by hyperstrophy from a dextral form, usually involving a planispiral intermediate phase, or third, by reversed symmetry from a dextral form. The developmental process of torsion, involving delayed appearance of the left compared with the right larval retractor muscle[8,9], predisposed primitive gastropods to dextral rather than sinistral coiling[10]. Hyperstrophic shells are characterised by an apex which is sunken below the apical edge of subsequently formed whorls, and by a base which protrudes as a false spire. Thus, while the shell is externally sinistral, the soft parts within are still dextral in organisation. Reversed symmetry

occurs when both shell and soft parts change in direction of coiling.

Excluding some slightly asymmetrical Bellerophontacea, very conservative estimates suggest that topographically sinistral coiling has independently arisen seven times among archaeogastropods (all but one lineage pre-Tertiary), four times among mesogastropods, six times among the marine neogastropods, twice in the Basommatophora (in one case leading to a major radiation of freshwater forms), and nine times among the land Stylommatophora (Table 1). An additional lineage contains primitive euthyneurans with sinistral larval shells together with the Thecosomata which retain sinistrality as adults. Within several lines of primitively sinistral freshwater Planorbidae, hyperstrophy has led to the evolution of pseudodextral coiling[13,14].

A necessary condition for the attainment of hyperstrophy appears to be the presence in the ancestral stock of an umbilicus, a cavity in the base of the shell created by the incomplete overlapping of adjacent whorls. Nearly all planispiral shells, most archaeogastropods, and most freshwater and land snails, are umbilicate; while most neogastropods and higher mesogastropods are not. It may be argued[15,16] that incomplete whorl overlap renders a shell more fragile than a similarly shaped non-umbilicate shell of equal thickness. In the sea, all but the smallest snails are potentially exposed to vigorous water movements and, especially in the tropics, to shell-breaking predators[17]. Small snails can often occur in the boundary layer at the water–substrate interface, where water movements are diminished, or live among algae or in other habitats where shell-breaking predators can find prey less effectively[1,17]. Hyperstrophy, involving a phylogenetic lowering of the true spire concomitant with an increase in umbilical width, should thus be rare among larger Recent marine gastropods because of an overall reduction in shell strength; but incipient hyperstrophy leading to a planispiral condition might still arise in small-shelled stocks.

In pre-Cretaceous seas, selection among large snails for strong shells may have been less intense than it is today since crabs and teleost fishes, which are among the most effective shell-breaking predators on Recent marine gastropods, were rare[18] or absent[19] before the Cretaceous. Data based on compilations from Knight *et al.*[20] suggest that about 74% of conispiral genera in the largely Palaeozoic Pleurotomariacea (Archaeogastropoda) have umbilicate shells, while calculations based on all known genera of Trochacea (Triassic to Recent)[20] and on all Recent North American genera[21] of this archaeogastropod superfamily yield estimates of 60% and 58% umbilicate genera respectively. The mostly non-umbilicate neogastropods and higher mesogastropods became important elements in the fauna only in the Mesozoic and especially the Cainozoic. Thus secondarily planispiral and hyperstrophic stocks could have evolved in pre-Tertiary times even among large-shelled gastropods.

The shells of many freshwater and land snails are fragile compared with those of marine forms. Many freshwater snails (notably Basommatophora) are annuals, and may adapt to predation by maintaining high reproductive rates rather than by developing impenetrable shells. This may also be true among many land snails, which in addition need not contend with water waves or currents. As in pre-Tertiary marine snails, selection against umbilicate shells is likely to be much reduced among freshwater and land snails, and hyperstrophy is more likely than among large Recent marine forms.

The taxonomic distribution of reversed symmetry may be explained partly by considering methods of larval dispersal. All 18–19 Recent lineages of gastropods with true sinistral coiling, except the Triphoridae and Thecosomata, develop without a planktonic stage. Since reversed symmetry becomes evident at cleavage[10], and as most mutations affecting events at such early developmental stages are associated with harmful side effects, the maintenance in a population of a mutant gene for reversed symmetry is unlikely except possibly in some small

Table 1 Ecological and geological distribution of planispiral (p), hyperstrophic (h) and sinistral (s) gastropods

Classification	p, h, and s taxa		Habitat	Age
Subclass				
Prosobranchia				
Order				
Archaeogastropoda				Cam.–Recent
Bellerophontacea	p	Whole superfamily	Mar. b.	Cam.–Tri.
Macluritacea*				Cam.–Dev.
Macluritidae	p	*Macluritella,*	Mar. b.	E. Ord.
	s?	*Omphaloceras*	Mar. b.	Dev.
Euomphalacea*				Ord.–Cret.
Euomphalidae	p	*Lesueurella, Amphiscapha, Planotectus*	Mar. b.	Ord.–Perm.
Weeksiidae	p	Whole family	Mar. b.	Cret.
Helicotomidae	p	*Ophiletina*	Mar. b.	M. Ord.
Pleurotomariacea				
Gosseletinidae	p	Gosseletininae	Mar. b.	Ord.–Tri.
Porcelliidae	p	Whole family	Mar. pl.?	Dev.–Jur.
Eotomariidae	s?	Agnesiinae	Mar. b.	Dev.–Tri.
Platyceratacea				
Holopeidae	s?	*Antitrochus*	Mar. b.	Dev.
Amberlyacea				
Cirridae	s?	Whole family	Mar. b.	Tri.–Jur.
Clisospiracea				
Clisospiridae	s?	Clisospirinae	Mar. b.	Ord.–Sil.
Trochacea				
Trochidae	s	*Calliostoma incerta*	Mar. b.	Recent
Turbinidae	h?	*Scaevola*	Mar. b.	Jur.
	p	*Helicocryptus, Pseudoliotina*	Mar. b.	Jur.–Cret
Order				
Mesogastropoda				Ord.–Recent
Valvatacea	p	Some Valvatidae	Fr.	Jur.–Recent
Viviparacea				
Pilidae	p	*Marissa cornuarietis*	Fr.	Recent
	h	[11]*Lanistes*	Fr.	Tert.–Recent
Atlantacea	p	Most Atlantidae	Mar. pl.	Cret.–Recent
Loxonematacea				
Zygopleuridae	s	*Virgella, Allostrophia*	Mar. b.	Tri.–Jur.
Rissoacea				
Hydrobiidae	p	Cochliopinae	Fr.	Recent
Vitrinellidae	p	Many genera	Mar. b.	Cret.–Recent
Omalogyridae	p	Whole family	Mar. b.	Recent
Triphoracea	s	Triphoridae	Mar. b.	Cret.–Recent
Cyclophoracea				
Cyclophoridae	s	Many *Diplommatina*	Land	Recent
Order				
Neritogastropoda				Dev.–Recent
Helicinidae	p	*Ceratodiscus*	Land	Recent
Neogastropoda				Cret.–Recent
Muricacea				
Buccinidae	s	Some *Volutopsius* and *Neptunea*	Mar. b.	Tert.–Recent
Melongenidae	s	Some *Busycon*	Mar. b.	Tert.–Recent

Classification	p, h, and s taxa		Habitat	Age
Conacea				
Conidae	s	*Conus adversarius*	Mar. b.	Mio.–Plio.
Terebridae	s	*Terebra inversa*	Mar. b.	Plio.–Quat.
Turridae	s	*Antiplanes*	Mar. b.	Recent
Subclass				
Euthyneura				
Order				
Entomotaeniata				Perm.?–Recent
Pyramidellidae	p	*Cyclostremella*[7]	Mar. b.	Recent
Order				
Thecosomata				Tert.–Recent
Limacinidae	h	Most Limacinidae	Mar. pl.	Tert.–Recent
	p	*Limacina inflata*	Mar. pl.	Recent
Peraclididae	h	Whole family	Mar. pl.	Recent
Order				
Basommatophora				Jur.–Recent
Ellobiacea				
Ellobiidae	h?	*Blauneria*[12]	Mar. b.	Recent
Ancylacea	s	Physidae, many Planorbidae and Bulinidae	Fr.	Tert.–Recent
	p	Many Planorbidae and Bulinidae	Fr.	Tert.–Recent
	h	Many Planorbidae[13,14]	Fr.	Tert.–Recent
Order				
Stylommatophora				Cret.–Recent
Achatinellacea	s	Many Achatinellidae and Partulidae	Land	Recent
Pupillacea				
Vertiginidae	s	Some *Vertigo*	Land	Recent
Chondrinidae	s	Some *Gastrocopta*	Land	Recent
Pupillidae	s	Some *Pupilla*	Land	Recent
Enidae	s	Some *Jaminia*	Land	Recent
Clausiliacea	s	Clausiliidae	Land	Recent
Corillacea				
Corillidae	h?	*Plectophylis*	Land	Recent
	p	Some *Corilla*	Land	Recent
Ariophantacea				
Ariophantidae	p	*Coxia*	Land	Recent
	s?	*Dyakia, Bertia, Ariophanta*	Land	Recent
Rhytidacea				
Rhytididae	p	*Diplomphalus*	Land	Recent
Endodontacea				
Endodontidae	p	*Hirasea*	Land	Recent
Zonitacea				
Zonitidae	p	Some *Helicodiscus*	Land	Recent
Systrophiidae?[22]	p	*Polygyratia*	Land	Recent
Polygyracea				
Polygyridae	p	Some *Polygyra*	Land	Recent
Ammonitellidae	p	*Ammonitella, Spelaeodiscoides*[22]	Land	Recent
Helicacea				
Camaenidae	s	Some *Camaena, Syndromus, Eulota,* and *Amphidromus*	Land	Recent

Mar., marine; b., benthic; pl., planktonic; Fr., freshwater; Cam., Cambrian; Tri., Triassic; Dev., Devonian; E. Ord., Early Ordovician; Perm, Permian; Cret., Cretaceous; Jur., Jurassic; Mio, Miocene; Plio., Pliocene; Quat., Quaternary.

* Most Macluritacea and many Euomphalacea are hyperstrophic, but their orientation and mode of life are poorly understood.

populations in which stabilising selection has been relaxed[6]. Left-handedness in normally dextral snails is often associated with aberrant shell shape[12,23-25]. The general reduction in dispersability associated with loss of a planktonic stage may lead to a higher probability of geographic isolation of small populations. The greater the number of such populations, the higher is the overall probability for reversed symmetry to arise. Reversed symmetry is thus most likely and most widespread in groups such as the Neogastropoda, containing many lineages with direct development, and especially the hermaphroditic and often locally distributed Stylommatophora.

Several anomalies in the distribution of gastropod coiling types are unexplained. All sinistral neogastropods occur or are inferred to have lived in marine soft-bottom environments, none being known from hard substrata. Among land Stylommatophora, sinistral coiling is rare in Africa, Australia and the Americas, but quite common in Europe and especially South Asia and the Pacific islands. The near absence of sinistral species in the three or four stocks of terrestrial prosobranchs, and the evolution of only one small lineage of planispiral forms in this group, are additional enigmata.

Intense stabilising selection may preclude the evolution of planispiral, hyperstrophic, and sinistral gastropods from dextral ancestors because the mechanisms altering coiling direction are normally selected against. This does not mean that all coiling types are adaptively interchangeable[6]. It has been suggested[12] that hyperstrophy in benthic snails renders crawling more difficult than in dextral or sinistral types. The benefit of improved locomotory efficiency with planispiral compared with asymmetrical coiling may in some cases out-

weigh the disadvantage of a weaker shell. Relaxation of selection for strong shells or for efficient locomotion could thus allow other, sometimes opposing, selective pressures to fix and maintain new coiling types.

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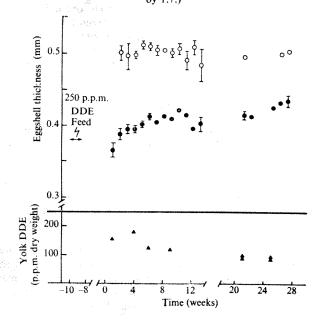
Prolonged eggshell thinning caused by DDE in the duck

ALTHOUGH eggshell thinning induced by 2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene (DDE)—has been studied in many species (see review by Cooke[1]), the long term effects of this pesticide have not been investigated. Its duration of action is of great environmental significance since it will determine the effect of any pesticide ingested before the breeding season. In this respect, the finding of continuing high levels of DDE and thin eggshells in the Alaskan peregrine falcon (Falco peregrinus) in areas free from direct spraying[2] argues for a prolonged effect, but direct controlled studies have been lacking. In fact, 18 d is the longest period to date during which thinning has been monitored (in mallards given single oral doses of 500–2,000 mg DDE kg^{-1}) and little recovery of shell thickness was observed[3].

We report here the changes in eggshell thickness in the white Peking duck (a domesticated mallard) during 27 weeks following a brief exposure to dietary DDE. Ducks approximately 6 months old were divided into experimental and control flocks (three birds in each) and maintained as before[4]. The experimental flock was fed 250 p.p.m. DDE for 10 d and then control duck feed (provided by Agway Inc.) for the rest of the experiment. A rough calculation from the rate of food consumption shows that experimental ducks (body weight 5–6 kg) ingested about 0.5 g DDE during the 10 d. Approximately 2 months later, when all birds began laying, eggs were collected daily and frozen, and the shells were cut at the equator, that is, approximately midway between the ends. Eggshells were washed in warm water and dried overnight at 45 °C. Twenty measurements of shell thickness, ten per half, were made near the equator of the egg using an Ames micrometer. Each bird produced an average of three or four eggs per week, except for a moulting period of about 7 weeks, when none was laid (weeks 14–20, Fig. 1). As Fig. 1 shows, eggshells from ducks fed DDE were approximately 20% thinner than controls, which agrees with the maximum thinning

reported before[4]. Recovery of shell thickness was slow, being less than half-way at the end of 27 weeks. DDE residues in the yolks were analysed as previously described[5]. Initially, levels were greater than 150 p.p.m. dry weight (Fig. 1) and after 27 weeks, residue values had declined only to about 90 p.p.m. In contrast, levels of DDE residues in a control egg yolk (from week 5) were 0.23 p.p.m. Based on the values of residues shown in Fig. 1, we have calculated the approximate amount of DDE lost by a bird through its eggs. Using a value of 150 μg DDE per g of dried yolk, and assuming that 75 eggs were laid during the 27 weeks, each containing 18 g of yolk (dried weight), roughly 0.2 g DDE or 40% of the original dose would be lost. This calculated clearance parallels the measured decrease in residue levels, that is, approximately 40% decrease in yolk DDE in 25 weeks (Fig. 1). Likewise, Tucker and Haegele[6] found significant loss of DDT through the eggs of mallards. Thus, in the duck, the egg is the major route of excretion for DDE. Naturally, in the wild, the loss would be much less since many fewer eggs are laid.

Earlier work on the white Peking duck demonstrated the rapid onset of eggshell thinning, which is essentially complete within 4 d of exposure to 40 p.p.m. of DDE in the diet[4]. Our study now shows that although the egg is a major route of excretion of DDE, loss through this mechanism is relatively slow, as is recovery of eggshell thickness. Thus, DDE-induced eggshell thinning has a rapid onset and is essentially irreversible.

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Fig. 1 Eggshell thickness as function of time for control (○) and DDE-fed (●) white Peking ducks. Experimental birds were fed 250 p.p.m. DDE only during the time indicated. Each point represents the mean thickness for all eggs collected during the 1 week (approximately 11 eggs per flock per week). Variability, when large enough, is shown as standard error bars. Also shown are DDE residues in randomly selected egg yolks from ducks fed DDE. Values are given as p.p.m. dry weight in individual egg yolks. (These values can be converted to p.p.m. wet weight by multiplying by 0.23 or to p.p.m. lipid weight by multiplying by 1.7.)

Acclimation in arctic lichens

USING a new gas exchange method, we have followed the seasonal response of net assimilation rate (NAR) in Arctic lichens and have found rapid acclimation to temperature, light and thallus moisture content. This suggests that these organisms can adjust reversibly their patterns of physiological response to suit the atmosphere of their rather stressful habitats.

Although there is increasing evidence of temperature acclimation of net assimilation rate (NAR) in various plants, both in the field[1-3] and as responses induced under laboratory conditions[4,5], investigations with higher plant systems are complicated by a combination of acclimatory response with variations of NAR resulting from seasonal production of new shoots, flowering and senescence of leaves. Lichens do not undergo seasonal growth of this sort and thus are an excellent system in which to study acclimation.

Infrared gas analysis can be used to measure NAR of plant tissue as a change in CO_2 concentration within a flowing gas stream before and after it passes over the experimental plant material, which is enclosed in a special assimilation chamber. We have found that the gas flow networks and environmental control systems which are necessary are too costly and complex to permit use of more than one or two assimilation chambers. Since only one replicate sample can be included in the chamber at once, any adequately replicated experiment is very time
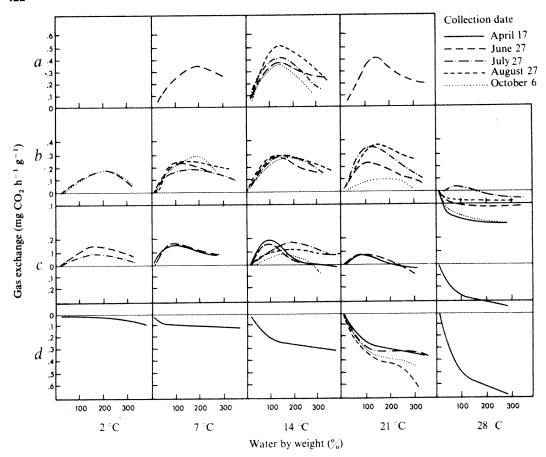
Fig. 1 Seasonal response of NAR in *Cetraria nivalis* Rates of assimilation (μeinstein m^{-2} s^{-1}): *a*, 250; *b*, 150; *c*, 50; *d* 0. Each line is the mean of four or five replicates with s.e.m. less than ± 0.02 mg CO_2 h^{-1} g^{-1}. All collection dates are from 1974.

consuming. Although such methods have been used to investigate gas exchange characteristics of lichens[3]–[5], the time required to study the basic responses to light, temperature and moisture has frustrated many attempts to demonstrate rapid acclimation in these plants[6,7].

We have conducted parallel experiments on various lichens from Arctic tundra using both a standard ventilated system[9] and a new method involving sealed gas exchange cells. We found that unlike the situation with higher plants, ventilation does not influence rates of CO_2 exchange by lichens, and that the concentration of CO_2 around the thallus may fall as low as 150 p.p.m. without any change in the rate of depletion of CO_2 from the vessel. Our results suggest that in these lichens, rates of net assimilation are so low that no strong CO_2 gradients appear within the thallus. It is also important that gas exchange can occur freely across the thallus since stomata are absent. Small samples of lichen (0.25 g) were placed in clear gas exchange cells, sealed and incubated under specified conditions of temperature and radiation within a walk-in growth chamber. After incubation for a specified time the change in CO_2 concentration within the gas exchange cells was measured by injection of a sample into a carrier gas stream flowing through an infrared gas analyser. The value obtained was standardised to yield a value of mg CO_2 h^{-1} g^{-1} dry weight of the thallus. This was plotted against the water content of the thallus—the primary controlling factor of carbon assimilation in lichens— which was measured by weighing before and after each incubation. Rates of net assimilation for *Cetraria nivalis* and *Parmelia caperata* found using this system were the same as those previously obtained by standard methods[4,8,10].

We have used our system to follow the seasonal response of NAR to three variables in *Cetraria nivalis* collected from Arctic tundra at East Pen Island, Hudson Bay. In Fig. 1 data appear as a 'physiological matrix' of responses to all thallus moisture levels, five temperatures and four light levels, measured five times during 1974. Basic gas exchange response changed con-

tinuously throughout 1974. The extent of these changes was considerable: for example levels of assimilation achieved at 250 μeinstein m^{-2} s^{-1} (1 einstein is 1 Avogadro's number (6.023×10^{23}) of photons, and 1 μeinstein is approximately equal to 50 lx) and 14 °C in June were only about 15% higher than at 150 μeinstein m^{-2} s^{-1}. Conversely, by July and August, levels of assimilation at 250 μeinstein m^{-2} s^{-1} were 60% higher than at 150 μeinstein m^{-2} s^{-1}. By October, after the first snowfall, the maximum NAR at 250 μeinstein m^{-2} s^{-1} was again only 15% higher than at 150 μeinstein m^{-2} s^{-1}.

At 50 μeinstein m^{-2} s^{-1} and 14 °C in April NAR in *Cetraria nivalis* was optimal at 100% water by weight; by July and August the response to thallus water content was much flatter, with an increase in NAR at higher levels of thallus water content. By October, the response had decreased and indicated a return towards the response found in the spring.

At 150 μeinstein m^{-2} s^{-1}, the optimal temperature for NAR was near 14 °C in June, near 21 °C in July and had shifted back towards 7 °C in October. In October NAR at 21 °C was about 70% of its value in late August.

It is not known whether the capacity for rapid acclimation demonstrated here is restricted to lichens collected from extreme environments such as East Pen Island or whether temperate or tropical species have equal facilities for acclimation. This could be tested by comparing ecotypes of species such as *Cladina mitis*, the distribution of which extends from high latitude sites like East Pen Island to temperate pine woodland. Although samples of this species collected in Wisconsin[11] showed no significant ecotypic or seasonal variation in response to light intensity, very different patterns can be expected from this species collected from tundra sites.

Our experimental system, with lichens as the experimental material, provides evidence that acclimation occurs very rapidly, is not restricted to shifts in temperature optima and may involve changes in response to light intensity and water content. We are investigating how the assimilation rate of lichens

acclimated to cold, dark and dry conditions is reversed after transfer to warm, bright and wet environments. On the basis of available evidence we suspect that such acclimation can be induced in a matter of days.

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Petroleum-degrading achlorophyllous alga *Prototheca zopfii*

ONE of the objectives of this research programme is to enumerate and identify petroleum-degrading microorganisms occurring in Chesapeake Bay, to assess the potential of these microorganisms to degrade petroleum *in vitro* and *in situ* and to determine the types of petroleum degraded[1]. We are sampling two locations in Chesapeake Bay at monthly intervals. One is Colgate Creek, an oil-contaminated site in Baltimore Harbour, the other is an area south of Parson's Island in Eastern Bay, a non-polluted shellfish harvesting region of Chesapeake Bay. The presence of petroleum in Colgate Creek and its absence in Eastern Bay has been confirmed by computerised low-resolution mass spectrometry analysis of benzene extracts of sediments collected at both sites[2,3]. We have routinely isolated petroleum-degrading bacteria, yeasts and filamentous fungi from Colgate Creek and Eastern Bay, but during April and May 1973, and again in 1974, an unusual organism, believed to be an algal species, was isolated from sediment of Colgate Creek. Corresponding isolates have not been isolated from Eastern Bay.

Routine environmental parameters measured during April and May 1973 and 1974 yielded similar data for both years:

air temperature 18–21 °C; dissolved oxygen, 9–12 p.p.m.; water temperature, 12–17 °C; pH 7–8, and salinity, 6–8 ‰. The unusual organism was isolated at a time of year when significant petroleum biodegradation occurred in Colgate Creek[4].

The organism was isolated, in axenic culture, on silica gel oil medium, supplemented with streptomycin and tetracycline which inhibited growth of petroleum-degrading bacteria[5]. The significant feature of our isolate is that it degrades petroleum hydrocarbons. When the organism was grown in 100 ml estuarine salts solution[1] overlaid with 1% (v/v) of a South Louisiana crude oil, we observed significant degradation of the oil, after 30 d of incubation at 25 °C in quiescent culture, compared with the uninoculated, weathered control sample of oil (Fig. 1).

The isolate has been identified as *Prototheca zopfii* (Table 1), following the description provided by Arnold and Ahearn[6], as well as by photomicroscopy (Fig. 2), and electron microscopy (Fig. 3), as recommended by Nadakavukaren and Mc-Craken[7] for differentiating *Prototheca* from fungi. As well as crude oil, the isolate utilised petroleum hydrocarbons in a 17-component mixed hydrocarbon substrate[8], after 14 d of incubation under the conditions described in Table 2. The mixed hydrocarbon substrate permitted determination of the
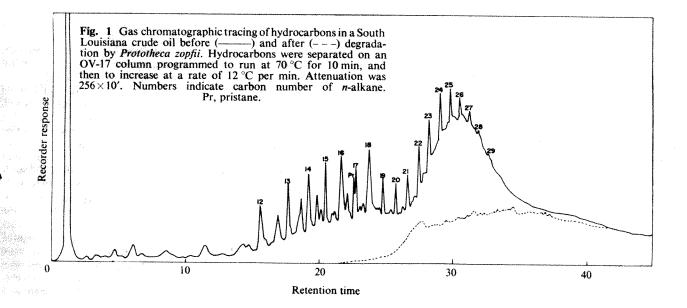
Table 1 Physiological characteristics of *Prototheca zopfii*

Assimilation of carbon compounds:

Glucose	+	L-arabinose	−
Galactose	+	D-arabinose	−
L-sorbose	−	D-ribose	−
Maltose	−	L-rhamnose	−
Sucrose	−	Glycerol	+
Cellobiose	−	*iso*-erythritol	−
Trehalose	−	Adonitol	−
Lactose	−	Dulcitol	−
Melibiose	−	D-mannitol	−
Raffinose	−	D-sorbitol	−
Melezitose	−	α-methyl-D-glucoside	−
D-xylose	−	Salicin	−
		Inositol	−

Assimilation of potassium nitrate: negative
Growth at 37 °C: positive
Growth at 10 °C: negative

Results of assimilation tests after 28 d incubation. Yeast nitrogen base (Difco) plus 0.5% carbon source. Yeast carbon base (Difco) plus 0.5% potassium nitrate. Growth at various temperatures: YM agar (Difco), 7 d.

Fig. 1 Gas chromatographic tracing of hydrocarbons in a South Louisiana crude oil before (———) and after (– – –) degradation by *Prototheca zopfii*. Hydrocarbons were separated on an OV-17 column programmed to run at 70 °C for 10 min, and then to increase at a rate of 12 °C per min. Attenuation was 256 × 10′. Numbers indicate carbon number of *n*-alkane. Pr, pristane.
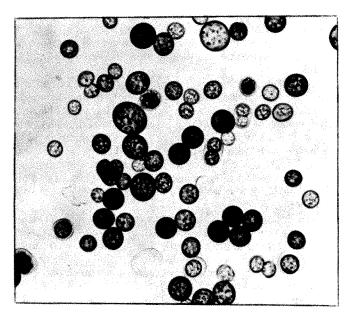
Fig. 2 Photomicrograph of the petroleum-degrading *Prototheca zopfii* (×750).
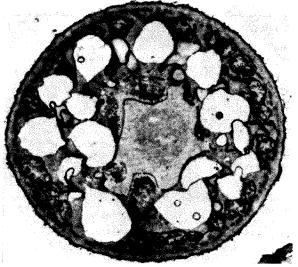
Fig. 3 Electron micrograph of a thin section through a *Prototheca zopfii* cell showing internal structures typical of this species[7]. A 48-h culture grown in 1% glucose-estuarine salts medium was fixed in 1% sodium permanganate for 8 h, dehydrated through a graded ethanol series, embedded in Epon, sectioned and poststained with uranyl acetate and lead citrate. The thin sections were examined and photographed with an RCA EMU-3F electron microscope (×9,500).

Table 2 Degradation of hydrocarbons in mixed hydrocarbon substrate by *Prototheca zopfii*

Hydrocarbon	% Remaining Weathered sample	Degraded sample	Individual hydrocarbon degraded (%)
Cumene	1.58	0.02	98.74
n-Decane	7.66	0.01	99.88
n-Undecane	7.59	0.46	93.94
Naphthalene	0.65	0.01	99.99+
n-Dodecane	7.76	2.33	69.98
n-Tridecane	8.20	4.06	50.49
n-Tetradecane	8.68	5.10	41.25
n-Pentadecane	11.28	7.37	34.67
n-Hexadecane	9.03	5.66	37.33
n-Heptadecane	13.58	8.62	36.53
Pristane	0.86	0.47	45.35
n-Octadecane	8.76	5.38	38.59
n-Nonadecane	8.45	5.02	40.60
n-Eicosane	10.82	6.50	39.93
Phenanthrene	0.41	0.24	41.47
1,2-Benzanthracene	0.47	0.28	40.43
Perylene	1.85	1.17	36.76

type of petroleum hydrocarbons utilised by this unusual algal isolate.

Growths of a *Prototheca* sp. on *n*-alkanes has been described[9], but this is the first report of the degradation of oil by an algal species. Although the significance of algae in this process has yet to be assessed, they were previously not considered capable of degrading petroleum hydrocarbons. The role of algae in the microbial degradation of oil must now be examined.

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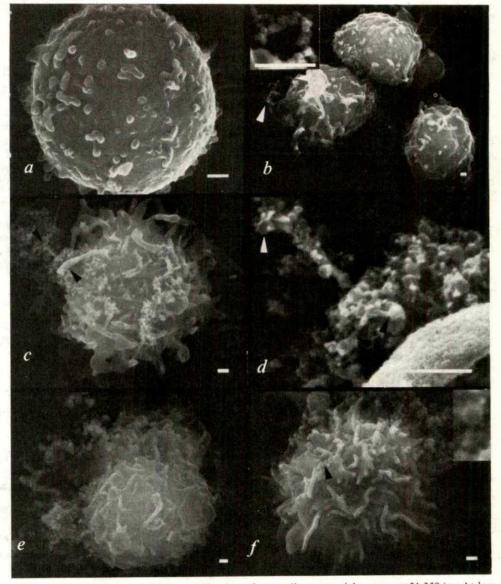
Multiple labelling technique used for kinetic studies of activated human B lymphocytes

IDENTIFICATION of distinct cell surface receptors which could be viewed in the scanning electron microscope would allow unambiguous identification of subpopulations of cells and quantitation of receptors on individual cells as well as permitting kinetic studies of the membrane changes following the binding of a ligand with its receptor. Labelling techniques using one or two markers have been developed for transmission electron microscopy (TEM)[1,2]. But scanning electron microscopy (SEM) offers several advantages over TEM for analysing membrane receptors in that it does not require serial sectioning of material[3] and therefore allows rapid viewing of the surfaces of large numbers of intact cells with a minimum of preparation. Here I describe new labelling techniques for SEM using three distinct markers conjugated to antibodies specific for the immunoglobulins IgA, IgG and IgM.

Previous labelling techniques developed for SEM[4-6] have several limitations. First the techniques use only one marker, restricting visualisation to only one type of receptor[4-6]. Second, with one exception[4], the techniques utilise a large, spherical (130–230 nm diameter) marker which obscures the cell surface preventing precise localisation and quantitative analyses of receptors. Third, the marker has a hydrophobic surface which makes it stick nonspecifically to many surfaces and molecules, and the absorbed protein can dissociate from the marker at physiological salt concentration and pH (ref. 6).

This study was undertaken to determine the feasibility of employing a tri-label technique for SEM. The SEM markers, which were selected for their size and distinctive shapes, were the cylindrical molecule, keyhole limpet haemocyanin (KLH), the icosahedral non-plaque forming simian virus 40 (SV40) and the hexagonal-headed T2 bacteriophage (T2). Antibodies specific for immunoglobulins (Ig) A, G or M were conjugated to individual markers[11], and human B cells carrying Ig were then selectively labelled with these reagents. Surface changes associated with activation were followed during kinetic studies to try to resolve the controversy concerning the appearance

Fig. 1 Lymphocytes were separated from heparinised human blood on "Lymphoprep" (Neygaard and Co.)[12], washed three times in a 1:1 mixture of the decomplemented, autologous serum and PBS, pH 7.4 and resuspended in Medium 199, pH 7.4, at a concentration of 1×10^6 cells ml^{-1}. Macrophages were removed by adherence to glass. Sheep red blood cell (SRBC) rosettes were formed by a modification of previous techniques[10,14]. Briefly, autologous heat inactivated serum, which had been absorbed against SRBC at 4 and 37 °C, was added to the lymphocyte suspension so that the final serum concentration was 20%, and the cells were incubated for 60 min at 37 °C. Washed SRBC were then added and the suspension layered on "Lymphoprep" at 4 °C and centrifuged at 400g for 15 min at 24 °C. The non-rosetting B lymphocytes which comprised about 20% of the total peripheral blood lymphocytes were collected from the top of the gradient, washed three times with Medium 199, and 0.1 ml pipetted on to 12 replicate glass coverslips inside plastic Petri dishes (Falcon). All three SEM marker conjugates were added to each coverslip. Controls consisted of unconjugated markers added to six coverslips. For kinetic studies, cells were incubated for 2 min at 37 °C or for 15, 30 or 45 min at 4 °C, fixed for 30 min with 1% GA and then for 4 h with 2.8% GA[10], dehydrated[10], CO_2 critical point dried, and coated with 5 nm of gold–palladium. Specimens were viewed for between 4 and 8 h in an Hitachi HHF 2R SEM operated at 20 kV. A minimum of 2,000 cells in each preparation were studied. *a*, Control untreated B cell. The surface is not labelled and is smooth except for microvilli. *b*, Elongated macrovilli are labelled with KLH-anti-IgA (between arrows; 2 min after label). Inset shows KLH as a rectangle when viewed sideways and as a circle when viewed on end (high magnification of area adjacent to right arrow ×31,350.) *c*, At low magnifications, T2-anti-IgM appears as 'fuzz' on the cell surface which is covered with elongated macrovilli (×5,225). A 'cap' has formed on the upper left of the cell (15 min after label). *d*, High magnification of the area between the arrows in *c* (×37,400) demonstrating T2 (black arrows indicate junction between the head and tail of T2). White arrow indicates another T2 of which both head and tail are visible. Because of the density of marker, generally only T2 heads or tails are visible as in the upper right of the micrograph. Apparently, the phage was osmotically shocked during the conjugation procedures because most of the heads are round rather than hexagonal. *e*, SV40-anti-IgG labelled B cell with undulate surface (15 min after label). *f*, B cell labelled with T2-anti-IgM at 30 min. Lamellae cover the surface, and the marker which is too dense to count has 'capped' on the upper part of the cell. Marker is also in scattered areas on the surface. Inset is high magnification (×75,900) of T2 from areas at the tip of the arrow. Magnification line equals 500 nm.

of human B cells under SEM. Previously, human B cells have been reported to be either highly undulate[8], highly villous[8,9] or sparsely villous[10], and this controversy has precluded the use of SEM as a simple method for distinguishing between B and T cells.

Markers were fixed in 1% glutaraldehyde (GA) for 2 h to preserve their structure and then dialysed overnight against phosphate buffered saline (PBS), pH 6.9. One ml of IgG fraction of goat antiserum directed against human IgM (3.4 mg ml^{-1}), IgG (15.2 mg ml^{-1}) or IgA (7 mg ml^{-1}) was added to 2 ml of T2 (Miles Lab), SV40 or KLH, respectively, so that the ratio of antibody molecules to marker was 1:1. The antisera were specific for the Ig against which they were made as determined by immunoelectrophoresis and double diffusion in agar. An 8% solution of GA was added dropwise with agitation until its final concentration was 0.1%. Marker labelled antisera preparations were then incubated at 20 °C for 2 h with agitation[11], and dialysed against PBS, pH 7.4, overnight at 4 °C. Conjugates were purified by column chromatography on Agarose A 1.5 m (Biogel 100–200 mesh). The first millilitre containing aggregates

was discarded. The conjugate was present in the first peak, unconjugated marker in the second, and unconjugated gamma globulin in the third.

In preliminary experiments, it was found that the amount of a marker present on a labelled cell was the same at a given incubation time irrespective of whether 1, 2 or 3 markers were used whereas the percentage of B cells labelled increased as more than one marker was used ($1 < 2 < 3$). The relative number of cells labelled with T2-anti-IgM, SV40-anti-IgG, or KLH-anti-IgA varied with the individual whose cells were tested (some had a preponderance of B cells carrying IgM while others had a preponderance of IgG cells). No cells were double labelled. When B cells were treated with a mixture of unlabelled and marker labelled anti-Ig, labelling was drastically reduced. These results confirmed the specificity of the marker-anti-Ig conjugates. "Stacking" of the marker was observed when conjugates were added to live cells but not when the marker was added to cells prefixed with 1% GA and washed in buffer. Moreover, aggregates were not observed when conjugates alone were scanned. This shows that the "stacking"

is not a result of the presence of aggregates in the conjugates. Rather it requires a viable cell membrane, suggesting that membrane fluidity may be responsible.

Incubation times and temperatures for the kinetic studies were selected on the basis of results from preliminary experiments using 1, 3, 6, 9, 12, 15 or 30 min at 37 °C, and 5, 15, 30, 45 or 60 min at 4 °C. All morphological changes observed with SEM occurred within 6 min at 37 °C which is sooner than can be detected with light microscopy. Since this was much too rapid to enable careful analysis of these cellular changes, most experiments were performed at 4 °C to slow membrane movement. It was found initially that cells incubated at 4 °C for 60 min, but not 45 min, showed subtle changes in surface ultrastructure including occasional contraction and collapse of some villi and lamellae; therefore 45 min was selected as the maximum incubation time at 4 °C.

B cells in the untreated control preparations had fewer than 100 microvilli per cell, evenly distributed on the surface (Fig. 1a). Within 2 min of addition of marker-antisera conjugates at 37 °C, the microvilli on labelled cells had migrated to one pole of the cell forming a 'cap', while the elongated macrovilli and lamellae formed at the other (Fig. 1b). An average of 10 markers were visible on each labelled cell. The inset on Fig. 1b shows clearly the marker, KLH, on the elongated macrovilli. KLH is also present on the 'capped' microvilli. By 15 min, elongated macrovilli and lamellae covered the surfaces of 70% of the B cells (Fig. 1c and d) and an average of 300 markers were visible on each labelled cell. Figure 1e shows an IgG-producing B cell labelled with SV40 anti-human IgG. Within 30 min, the surfaces of 50% of the labelled B cells were extensively covered with lamellae, and the marker had again 'capped' (Fig. 1f). No difference was observed in the surface appearance of IgA, IgG and IgM cells. When kinetic experiments were repeated using unconjugated Ig, the same sequence of membrane changes were observed indicating that these are not artefacts resulting from binding of a heavy marker.

These results indicate that unstimulated B cells have microvilli on their surface but do not have elongated macrovilli or lamellae. When activated by anti-Ig receptor reagents, B cells respond first with the formation of elongated macrovilli and then lamellae. Simultaneously with the appearance of macrovilli, the number of Ig receptors present on the surface increases by at least tenfold (from approximately 10 to 300 receptors per cell). This is a minimum estimate because (a) only 1/2 to 3/4 of any individual cell can be seen at any one time with SEM, (b) the marker may be bound in layers or may have entered the cell and (c) some of the marker may have been removed during critical point drying. In spite of these limitations, SEM analysis of surface events, and binding of labelled molecules in particular, is more accurate than TEM analysis which depends on the plane of section (that is, a typical 30 to 60 nm section of a cell may or may not be through an area where the receptors are present in a proportion representative of the whole cell).

Some authors have shown previously that human B cells have relatively few microvilli[3] while others have contended that they have highly villous[12] or undulating surfaces[1]. As is evident from the results of these experiments, the surface appearance of B cells depends on their stage of activation. Those who described B cells as having relatively few villi had incubated them in PBS without antibody or complement[10,13], whereas those who described them as "highly villous", used non-autologous pooled Type AB serum which had not been previously absorbed with the lymphocytes used in the experiment[8,9]. Those who described B lymphocytes as "villous" and "highly" undulate had incubated lymphocytes for 60 min at 0 °C with complement-coated human red blood cells[7]. These conditions can activate B cells and initiate the surface changes described. Although it might seem that these investigators were each describing a different type of cell, the results of the present study would indicate that they were actually describing different stages of activation of B cells. This suggests that conditions for

evaluating peripheral blood lymphocytes as 'T' or 'B' should be standardised.

A study of mouse T and B cells obtained from lymph nodes, incubated in basal salt solution, and fixed before addition of antisera conjugated to latex spheres by the method of Lo Buglio[6] agrees with our first report that human T cells have highly villous surfaces while B cells had relatively few microvilli[10]. It seems that surface characteristics of unstimulated T and B cells of mice and men are remarkably similar.

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Regulation of cell size in fish of tetraploid origin

ALTHOUGH what precisely determines the characteristic size of the cell is still unknown its regulation must have been of enormous significance during evolution[1,2]. Bigger cells have a relatively lower metabolic rate than smaller ones so that the reduction of cell size is of adaptive value when changing environmental conditions requires an increase in metabolism and *vice versa*[1,2]. A decrease in cell size, however, must be accompanied by a corresponding loss of DNA if the nucleo-cytoplasmic relationship, which is fundamental in cell biology, is to be maintained.

We have reported on phylogenetically tetraploid Cyprinid fish, which still keep the tetraploid genome, but exhibit a reduction of cell size, compared with the expected value in tetraploid cells[3]. This reduction cannot be ascribed to loss of nuclear DNA. We therefore postulated a regulatory mechanism which acts at the level of specific genes. It is known that fish cell size, to some extent, parallels the amount of $(28S+18S)$ ribosomal genes[4]. In a group of closely related plant species the number of ribosomal genes is also reduced with increasing DNA content[5,6]. Since ribosomes are required for the translation of all RNAs specified by structural genes, a loss of ribosomal genes during the evolution of the tetraploids might well be responsible for a lower synthetic output of the cell, and, in consequence, for the reduction of cell size. In the present investigation we have determined the number of $(28S+18S)$ rRNA genes in the diploid and the phylogenetically tetraploid Cyprinid fish species (Fig. 1).

Surprisingly, a clear-cut 1:2 ratio of the number of $(28S+18S)$ ribosomal genes exists between the diploid and the tetraploid group amounting to a mean number of 272 and 544

Table 1 Cellular parameters in diploid and phylogenetically tetraploid Cyprinid fish species

	2n				4n		
	Rutilus rutilus	Tinca tinca	Abramis brama	Leuciscus cephalus	Barbus barbus	Cyprinus carpio	Carassius auratus
DNA content per nucleus (% of human leukocytes)[7]	28	30	36	38	49	50	53
Erythrocyte volume (μm^3)	202.0	203.8	182.0	186.0	190.8	181.6	180.6
Soluble protein content per erythrocyte (pg)	60.1	70.4	63.0	66.7	70.8	64.7	64.9
Nucleolar area (% of nuclear area)	3.7±0.6*	3.4±0.3	3.5±0.6	3.7±0.4	2.1±1.0	1.6±0.4	1.5±0.2

The erythrocyte volumes were determined with the haematocrit method and the protein content was measured according to Lowry et al. (for further details see Schmidtke and Engel[3].). The relative nucleolar size in 20 cells of each species was determined from smears of fin epithelial cells fixed in Carnoy, and stained with acridine orange.
*Mean ±s.d.

respectively. Although heterologous (28S + 18S) rRNA from HeLa cells and not homologous rRNA from fish cells was used, the observed values approximate the true number of ribosomal genes in the Cyprinid fish species very closely. When DNA of Carassius auratus was hybridised with homologous (28S + 18S) rRNA a saturation value of 0.00054 (rRNA/DNA) was observed[4]; using HeLa rRNA we found a mean saturation value of 0.00052 in this species. These results imply far-reaching homology between fish and human (28S + 18S) rRNA cistrons.

It is clear that in the diploid–tetraploid system dealt with in this investigation there is no correlation between cell size and the number of (28S + 18S) rRNA genes. It could be assumed, however, that, referring to the expected tetraploid cell size, the reduction to the diploid level was achieved by

diminishing activity of the ribosomal genes during evolution nucleolar size measurements point in this direction (Table 1) The diploid and the tetraploid Cyprinids have nucleoli of very similar size, which suggests similar synthetic rates of rRNA. This is being studied at present.

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Fig. 1 The number of (28S + 18S) rRNA cistrons per diploid genome in diploid and phylogenetically tetraploid Cyprinid fish species; a, Rutilus rutilus; b, Tinca tinca; c, a natural hybrid from Rutilus rutilus and Abramis brama; d, Abramis brama; e, Leuciscus cephalus; f, Barbus barbus; g, Cyprinus carpio; h, Carassius auratus. Each column represents the mean of about five specimens. DNA was isolated from erythrocytes lysed with Triton X-100, followed by treatment with Pronase and a SDS-concentrated saline mixture, several extractions with phenol, in the presence of m-cresol and 8-hydroxychinoline, and chloroform, and precipitation with isopropanol. Alkali-denatured DNA was loaded on Sartorius nitrocellulose membrane filters (35 μg per filter). DNA was hybridised at 65 °C in 6 × SSC-50% formamide essentially as described by Birnstiel et al.[8] with [3]H-labelled (28S + 18S) rRNA isolated from ribosomes of HeLa cells as described by Bross and Krone[9]. Specific radioactivity ranged from 40,000 to 50,000 c.p.m. μg[-1]. Saturating RNA concentration (> 5 μg ml[-1]) and saturating time course (4 h) were determined experimentally. The calculation of the number of 28S and 18S ribosomal genes was based on a molecular weight of fish rRNA of 1.51×10^6 and 0.7×10^6 respectively[10], and a diploid DNA-content of the human genome of 7.3×10^{-12} g (ref. 11).

Abnormal development of preimplantation rat eggs after three days of maternal dietary zinc deficiency

Dietary deficiency of zinc in pregnant rats causes congenital malformations of high incidence in multiple organ systems[1,2]. These teratogenic effects of zinc deficiency seem to be related to impairment of nucleic acid synthesis[3-6]. We now report that dietary zinc deficiency in the first few days of pregnancy results in abnormal cleavage and blastulation in preimplantation eggs, as early as day 3 of gestation in the rat.

Virgin female Sprague–Dawley rats were mated and fed a purified diet, either complete (100 p.p.m. Zn) or zinc-deficient (< 0.4 p.p.m. Zn), from the beginning of pregnancy. The day of finding sperm in the vaginal smear was considered day 0 of gestation. Diet composition and details of environmental control have been published previously[2]. Females were chloroformed on day 3 of gestation, the reproductive tract was removed, and the oviducts were isolated and flushed with saline introduced into the fimbriated ostium and collected in a watch glass from the distal end. On day 4 of gestation the uterine horns of anaesthetised females were distended slightly with calcium-free Krebs–Ringer solution containing 0.002% collagenase and 0.002% hyaluronidase introduced by cannula through an incision near the vaginal end. A ligature prevented escape of this fluid. Fifteen minutes later, the reproductive organs were removed to a saline bath, each horn was flushed from the vaginal end through a small incision near the tubo-

Table 1 Preimplantation rat eggs on day 4 of gestation as affected by maternal dietary zinc deprivation

Group	Females No.	Corpora lutea No.	Eggs Flushed		Normal Morula		Normal Blastula		Uncleaved		Abnormal Morula		Abnormal Blastula	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control (100 p.p.m. Zn)	9	131	101	78*	0	0	99	98†	2	2†	0	0	0	0
Deficient (<0.4 p.p.m. Zn)	12	142	113	80*	5	4†	19	17†	9	8†	34	30†	46	41†

$*\dfrac{\text{Embryoblasts recovered}}{\text{No. of corpora lutea}} \times 100.$

$†\dfrac{\text{Type of embryoblast}}{\text{Total no. of embryoblasts}} \times 100.$

uterine junction, and the flushings were collected in watch glasses. The procedure yielded approximately 80% of the anticipated embryos, as based on correlation of the number of recovered eggs with the number of corpora lutea in the corresponding ovary (Table 1).

Cleaving eggs obtained by this method responded normally to supravital staining, retained the zona pellucida for more than 7 h in normal saline at pH 7.4[7], and seemed to be identical morphologically to a small number of samples obtained from deficient and control animals by the methods of Nicholas[8]. Specimens were examined and photographed in saline.

In tubal flushings from seven control rats on day 3 of gestation, all eggs were in the eight or 16 cell stages (Fig. 1 *a* and *b*). In 11 zinc-deficient rats, 68% of the eggs on day 3 of gestation were in the eight-cell stage and appeared to be normal. However, the remaining eggs had either failed to cleave and were undergoing necrosis with disruption and fragmentation of the cellular contents, or consisted of three to seven blastomeres lacking normal orientation and showing variation in size (Fig. 1 *c–f*).

On day 4 of gestation, only two of 101 eggs recovered from control females had failed to undergo cleavage, while all remaining eggs were in the blastocyst stage (Table 1 and Fig. 2 *a* and *b*). Eggs from three of 12 zinc-deficient females were normal morulae or blastulae; all remaining preimplantation embryos from zinc deficient females were abnormal (Table 1 and Fig. 2 *c–f*).

These results clearly show that dietary zinc deficiency in the pregnant rat rapidly produced abnormal development of preimplantation embryos. This is the first time to our knowledge that abnormal development of cleaving eggs has been shown to result from a nutritional deficiency of the maternal diet. It is generally thought that embryos are insensitive to environmental influences before implantation and that exogenous teratogens are either lethal or without lasting effect during this period[9,10]. In the case of zinc deficiency in rats given a deficient diet only from day 0 to day 5 of gestation, preimplantation losses were minimal, and foetal malformations at term were only slightly increased over those of controls[2]. Thus it is possible that the morphologically abnormal blastocysts described here may recover to develop normally but this question remains to be answered.

The uterine fluid of rabbits has recently been found to contain significant amounts of zinc, approximately twice the concentration of the free-lying blastocysts[11]. Uptake of zinc by uterine fluid was, however, slow as compared with that of other reproductive tract components such as the endometrium[11]. It is therefore possible that with a maternal dietary deficiency of zinc, the milieu of the conceptus (the oviductal and uterine fluids) becomes inadequate in zinc as a consequence of competition with tissues having higher avidity for available zinc supplies in the face of the low maternal plasma zinc levels, which are known to occur[12]. The finding of abnormalities in cleaving eggs on day 3 of gestation suggests that intraluminal zinc may be reduced to critical levels before this time under these conditions.

Recent work has shown that cleaving mammalian eggs

Fig. 1 Examples of embryos obtained from the oviducts of control (*a* and *b*) and zinc deprived (*c–f*) female rats on day 3 of gestation. Note the presence of an intact zona pellucida in all examples. All embryos of both control and deficient dams were stained successfully with neutral red following photography. *a*, Control, eight-cell stage; *b*, control, sixteen-cell stage; *c*, abnormal embryo with three large intact blastomeres and the cellular debris of a fourth; *d*, abnormal seven-cell embryo containing one excessively large blastomere; *e*, abnormal embryo, cell boundaries lacking between blastomeres giving the appearance of a syncytium; *f*, abnormal embryo, showing derangement of orientation and great variation in size of blastomeres.

Fig. 2 Examples of embryos obtained from the uterine horns of control (*a* and *b*) and zinc deprived (*c–f*) female rats on day 4 of gestation. Note the presence of an intact zona pellucida in all examples. All embryos of both control and deficient dams were stained successfully with neutral red following photography. *a*, Control, early blastula stage; *b*, control, normal blastula; *c*, abnormal morula showing variation in blastomere size; *d*, early, blastocyst from zinc deficient female showing derangement of blastomeres and beginning of blastocoel (×); *e*, abnormal blastulation with dissociated blastomeres and a degenerating mass of cytoplasm. Blastocoel indicated by arrow; *f*, abnormal, lateral continuation of blastocoel cavity (arrow).

incorporated labelled precursors of DNA and RNA[13] and that mitotic inhibitors or compounds which suppress RNA synthesis adversely affected blastogenesis[14-16]. Thus, the abnormal development of preimplantation eggs reported here may result from impaired synthesis of nucleic acids, a zinc-requiring process.

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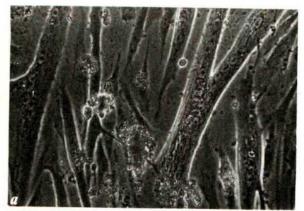
Temperature-sensitive expression of differentiation in transformed myoblasts

NUMEROUS studies have dealt with the replication of tumour viruses in fibroblasts, but little is known about the mechanisms by which such viruses interfere with the control of specific functions in fibroblasts or in differentiating cells. It is known that tumour viruses can transform cells from differentiated tissues[1-7]. Chick embryo myoblasts can be transformed by Rous sarcoma virus (RSV), (refs 2 and 3); transformation, or at least infection, prevents neither formation of myotubes nor the biochemical changes which ordinarily follow myoblast fusion[8]; Easton and Reich did not, however, purify infected cells by serial passage. We report the *in vitro* transformation of chick embryo myogenic cells with a temperature-sensitive mutant of RSV. We show that transformed myoblasts, after serial passage, lose their ability to differentiate at the permissive temperature (36 °C) but will differentiate to form myotubes—if transformation is blocked by a shift to the non-permissive temperature (41 °C).

Chick myoblasts derived from thigh muscles of 11-d-old chick embryos were infected with the Schmidt–Ruppin *ts*68 strain of RSV (ref. 9) and incubated at 36 °C (permissive temperature). On day 2 after infection, the presence of myotubes could be detected in all the Petri dishes with no obvious differences between mock-infected cultures and RSV-infected cultures. On day 3 after infection, there was still no difference between the mock-infected cultures and RSV-infected cultures. On day 4 after infection, some clumps of rounded cells began to appear in the infected cultures.

In the mock-infected chick embryo myoblasts (Fig. 1*a*) after 5 d in culture, most of the mononucleated cells have been replaced by long myotubes, each containing numerous nuclei. There are also a number of mononucleated cells which are believed to be fibroblasts. These cells form a monolayer and are never found in clumps or large aggregates. In the RSV-infected cultures after 5 d (Fig. 1*b*) the number of myotubes in the plate is reduced and many aggregates of rounded cells are found; most of these are attached to myotubes. This situation is in good agreement with results described previously[2,8], although it is not clear whether all the rounded cells are transformed cells or clumps of dead cells. It is likely that these rounded cells are indeed transformed cells, since no such cells ever appeared in RSV-infected cultures maintained at 41 °C (Fig. 1*c*). We also found, as did Easton and Reich[8], that infected myoblasts will form myotubes and that these myotubes are not stable and will disappear on further incubation.

The clumps of rounded cells (Fig. 1*b*) were allowed to grow for a few more days, then dispersed with trypsin and distributed into new Petri dishes. The medium was changed to a mixture of Dulbecco's modified Eagle's MEM and Medium 199 (5:2); 10% foetal calf serum and 1% chick embryonic extract were also
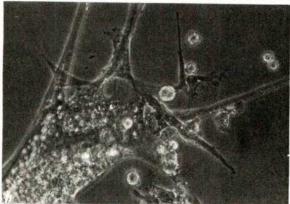
Fig. 1 Phase contrast photographs of chick embryo myoblasts infected with RSV *ts*68. *a*, Mock-infected culture on day 5 after infection. *b*, Infected culture maintained for 5 d at 36 °C; clumped cells have appeared. *c*, Infected culture maintained for 5 d at 41 °C. Chick embryo myoblasts were prepared by a modification of Konigsberg's method[16]. Thigh muscles from 11 day embryos, dissected free of skin and bones, were minced with scissors and incubated at 37 °C in phosphate-buffered saline (PBS): NaCl 137 mM, KCl 2.6 mM, Na_2HPO_4 8.1 mM, KH_2PO_4 1.4 mM for 15 min. The fragments were dispersed by pipetting through a Pasteur pipette and the dispersed cell suspension was centrifuged at 500g for 1 min. The upper nine-tenths of the supernatant was removed, diluted with complete medium (Dulbecco's modified Eagle's MEM containing 10% horse serum and 2% chick embryonic extract) and preplated at $5-10 \times 10^6$ cells on gelatin-coated Falcon tissue-culture Petri dishes (100 mm) to remove fibroblasts. (This procedure usually yields a 90% or better pure myoblast population.) After 15–20 min at 37 °C in a 5% CO_2 incubator, the unattached cells were transferred to gelatin-coated tissue culture Petri dishes (100 mm) at a plating density of 2×10^6 cells and the plates were incubated at 37 °C. Five hours later the cells were washed once with PBS and infected with 2 ml of RSV *ts*68 containing 10^6 focus-forming units ml^{-1}. After adsorption for 60 min at 36 °C, the cells were overlaid with complete growth medium and incubated at 36 °C in 5% CO_2 in air. Many multinucleated myotubes can be seen.

present. The cells were serially passaged with a starting density of 5×10^5 cells per tissue-culture Petri dish (100 mm) and trypsinised when the plates reached confluency (about $8-10 \times 10^6$ cells per plate). During these manipulations, one of the dishes was accidentally left too long and became overcrowded. The sheet of cells detached from the dish and the remaining cells were overlaid with fresh medium. A few clones had grown after a further 10 d; three of them (cl-1, cl-2, cl-3) were picked and further maintained in culture. After about ten generations, one Petri dish of each of the three clones and one Petri dish of the uncloned parental culture were shifted to the non-permissive temperature (41 °C). A change in the morphology of the cells was evident, 24 h after the shift, in all Petri dishes. The cells were more flattened and more dispersed, and some cells had lost the spindle shape characteristic at 36 °C. Figure 2*a* illustrates the changes which occurred after 3 d at 41 °C in both the uncloned parental culture and in cl-3. Numerous myotubes could be seen among a layer of mononucleated cells. In cl-1 and cl-2, no such myotubes could be seen even after a longer period of time at 41 °C.

On the other hand, at 36 °C, no formation of myotubes could be observed in any of the cultures. A more quantitative study, using the parental uncloned culture, is presented in Table 1. It is clear that after the shift to 41 °C there is a change in the overall composition of the population. Whereas at 36 °C, 99% of the cells remain mononucleated, after the shift up to 41 °C, 60% of the cells become polynucleated. It is, however, striking to observe that only 12% of these polynucleated cells contain more than ten nuclei as compared with 71% when primary chick embryo myoblasts are used (Table 1). This was taken to reflect the fact that the RSV-transformed myoblast population contains fewer cells which are able to fuse than do the primary myoblast populations. From this experiment it is clear that RSV-transformed myoblasts can be isolated and that these myoblasts, after serial passage, are prevented from further differentiation. Nevertheless this function is not lost, since following a shift up to the non-permissive temperature they will regain this function and will differentiate to form myotubes.

The nature of the cells which do not fuse at 41 °C remains to be elucidated. The most likely explanation is that these are transformed fibroblasts since the starting primary culture consists of 90–95% myoblasts and 5–10% fibroblasts. One must also consider that some of the mononucleated cells are transformed myoblasts which have definitively lost their ability to differentiate since clones of myogenic cells which do not fuse have already been described[12] (N. Teng, unpublished). A clear demonstration of either one or both of these hypotheses will require the isolation of morphologically pure clones of cells.

Finally, there is a striking difference between the myotubes which appeared after infection and the ones which were formed after the shift to 41 °C, since the latter are very stable and remain on the dish as long as would myotubes in an uninfected culture.

Fig. 2 Morphology of transformed myoblasts at 41 °C. Photomicrograph of the uncloned parental culture maintained for 3 d at 41 °C.

Table 1 Myotube formation by RSV-transformed myoblasts at 36 and 41 °C

	% Mono-nucleated cells	% Polynucleated cells 2–3 nuclei	4–10 nuclei	>10 nuclei
RSV-transformed myoblasts at 36 °C	99	1		
RSV-transformed myoblasts at 41 °C	40	12	36	12
Primary myoblasts at 36 °C	8	3	18	71

RSV-transformed myoblasts were seeded at 36 or 41 °C Primary chick embryo myoblasts, prepared as described in the legend to Fig 1*a*, were incubated at 36 °C Four days after seeding, one Petri dish of each culture was fixed and stained with haematoxylin (A minimum of 500 nuclei were counted in each plate)

In conclusion, we have shown that it is possible to isolate transformed chick embryo myoblasts in which the expression of differentiation is under the control of a viral gene It is too soon to make any speculation about the mechanism of control, especially since nothing is known as yet about the other changes which usually follow the event of fusion such as myosin synthesis[11], appearance of cholinergic receptors[12,13] and regulation of various enzymes[14,15]

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Diffusible mating-type factors induce macrocyst development in *Dictyostelium discoideum*

IN cellular slime moulds the formation of multicellular macrocysts constitutes an alternative pathway of development to that of fruiting bodies In appropriate conditions macrocysts form in certain strains of *Dictyostelium* and *Polysphondylium*[1] The existence of mating types in cellular slime moulds was suggested when certain combinations of amoebae of different strains resulted in macrocyst development[2,3] Since the aggregation of cells which leads to fruiting body formation in *D discoideum* is mediated by cyclic AMP[5,6], we decided to investigate whether the interaction between mating types is also mediated by a small, diffusible molecule Our results indicate that such a diffusible

mating factor does indeed regulate macrocyst formation in *D discoideum*

Stock cultures of two complementary, haploid mating types of *D discoideum* (strains NC-4 and V-12, given by Dr D W Francis) were maintained on *Escherichia coli* (strain B/r) on 0 2% Cerophyl agar These strains can from macrocysts when mixed but not when cultured separately[3] The general macrocyst-forming conditions of Nickerson and Raper were used in all experiments[7]

In the first set of experiments, a suspension (5 0 ml) of bacteria and spores of one strain of *D discoideum* in distilled water was pipetted into sterile dialysis tubing (pore size 4 8 nm) The tubing with ends tied was placed in a culture plate An equal volume of spore suspension of the opposite strain (experimental) or the same strain (control) was distributed uniformly over the tubing and the agar The plates were stored in light-tight metal cannisters at 23 ± 1 °C In every experiment separate cultures of V-12 and NC-4, which never produced macrocysts, and a mixture of V-12 and NC-4, which always produced macrocysts, were run as controls Macrocyst formation was assessed by microscopic (× 25–50) examination of plates after 7 and 14 d Whenever macrocysts formed they conformed in structure to that described before[1,2-4,7]

Table 1 summarises the results of these experiments When NC-4 was placed in the dialysis tubing and V-12 was outside, macrocyst formation was induced in eight out of 21 cultures Many macrocysts formed along the outside of the dialysis membrane, but few formed further than a few centimeters from the tubing The reverse combination, with V-12 inside the tubing and NC-4 outside, yielded no macrocysts Under the conditions used either macrocysts developed fully and were evident after 7 or 14 d, or no development began Small numbers of fruiting bodies appeared on all culture plates Thus cell contact between opposite mating types of *D discoideum* is not essential for macrocyst formation

Table 1 Induction of macrocysts across a dialysis membrane

Experimental situation	No of cultures	No of cultures with macrocysts NC-4	V-12
NC-4 alone	9	0	NA
V-12 alone	9	NA	0
(NC-4) × NC-4	7	0	NA
(NC-4) × V-12	21	0	8
(V-12) × NC-4	18	0	0

When dialysis tubing was used the cells contained within the tubing are listed first within brackets NA, not applicable

A second series of experiments was carried out to determine whether the continual presence of the opposite mating type was essential for macrocyst induction Each strain was cultured separately in macrocyst-forming conditions After 18 h, the cells were dislodged from the agar surface with a bent glass rod and the suspension was centrifuged at 2,000*g* for 5 min to pellet the whole cells The supernatant was filtered through a sterile Millipore filter and the final sterile, cell-free supernatant (conditioned medium) was poured over a fresh culture (0 h culture) of the opposite strain After 7 or 14 d, cultures were examined for the presence of macrocysts For controls, the supernatant of one strain was added to cells of the same strain and distilled water instead of supernatant was added to individual cultures of each strain

Table 2 shows that NC-4 supernatant induced macrocyst formation in V-12 66% of the time, but the reverse combination never resulted in macrocyst formation These data agree with the results of the first set of experiments and emphasise that cell contact between mating types is not essential for complete macrocyst formation Furthermore, the data indicate that a stable inducing factor (molecular weight about 12,000) was released by NC-4 very early under macrocyst-forming conditions The fact that V-12 supernatant did not induce a response in NC-4 cells suggests that this factor was unstable or possibly non-

Table 2 Effect of extracellular medium on macrocyst induction

Experimental situation	No of cultures	No of cultures with macrocysts
NC-4 supernatant to NC-4 cells	6	0
V-12 supernatant to V-12 cells	4	0
NC-4 supernatant to V-12 cells	35	22
V-12 supernatant to NC-4 cells	25	0
Distilled water to NC-4 cells	13	0
Distilled water to V-12 cells	13	0

existent The ability easily to induce V-12 to form macrocysts with NC-4 supernatant provides an excellent bioassay system for use in the purification of the mating factor

Macrocyst development might be divided into two events aggregation of single cells to form multicellular clumps, followed by differentiation of the aggregates into mature macrocysts It is unlikely that cyclic AMP is the small molecule that is involved in the formation of macrocysts of *D discoideum*, for fruiting bodies appeared in isolated cultures of NC-4 and V-12 when macrocysts could not form Furthermore, it seems that once macrocyst aggregation is induced, the events of differentiation must also be triggered since macrocyst development always went to completion

Although our results suggest that one strain (NC-4) could be an initiator or dominant strain in a sequential induction system, it is possible that the inducing factor from the opposite mating type (V-12) is simply less stable under our conditions

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Effects of progesterone and EDTA on cyclic AMP and phosphodiesterase in *Dictyostelium discoideum*

WHEN *Dictyostelium discoideum* amoebae are nutritionally deprived, they undergo a developmental programme which results in the formation of fruiting bodies composed of spore and stalk cells Early events in this cycle are marked by the aggregation of previously individual amoebae During this process they become elongated, and establish specific intercellular contacts, resulting in the formation of cell streams[1] Orientation of cell movement during this period is thought to be directed by an external gradient of cyclic AMP (ref 2) This compound elicits a chemotactic response from starved cells but not from growing cells[3,4] and can induce a period of coordinated movement in nutritionally deprived cells[5] A phosphodiesterase is also excreted by aggregating cells[6,7] activity of which modulates the cyclic AMP gradient Thus, a general model for communication between cells during aggregation is available Little is known, however, about the regulation of metabolic events which result in the differentiation of growth phase cells into aggregation-competent, cyclic AMP-sensitive cells, and in particular, the influence of cyclic AMP on these processes Since in other eukaryotes, changes in internal cyclic AMP are thought to mediate the actions of a variety of hormones, and regulate cell growth and
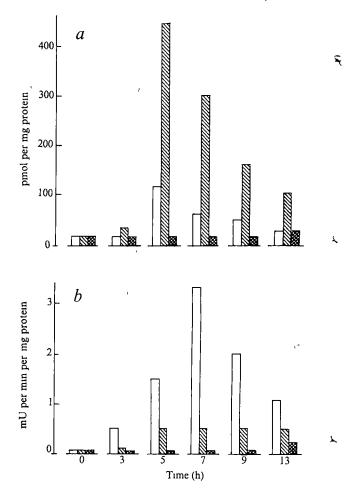
Fig 1 Intracellular cyclic AMP (*a*) and phosphodiesterase (*b*) levels (which includes both cytoplasmic and membrane-bound activities) in *D discoideum* cells incubated at 22 °C in buffer (open columns), buffer plus 10^{-3} M EDTA (hatched columns), buffer plus 3×10^{-5} M progesterone (cross-hatched columns) $1\ 4 \times 10^7$ Ax-2 amoebae, an axenic haploid strain[9], were plated on to 100×20 mm Falcon tissue culture dishes in 5 ml mM buffer [5 mM 2-(N-morpholino)-ethane sulphonic acid, pH 6 2, 10 mM KCl, 5 mM $MgCl_2$] with or without EDTA or progesterone[8] For studies in which cyclic AMP was measured, the media from four plates was aspirated at the indicated times and cells were immediately harvested in 5% cold TCA Samples were further prepared by the procedure of Maganillo and Vaughan[11], except that Dowex chromatography was performed at pH 6 Aliquots were assayed at three dilutions for cyclic AMP using the method of Gilman[11] For measurement of phosphodiesterase activity, cells were harvested in their media, centrifuged at 3,000g for 2 min and washed once with 50 mM Tris, pH 8, 4 mM $MgCl_2$ The pellet was frozen, then thawed in 1 ml 50 mM Tris, pH 8, 4 mM $MgCl_2$ and dialysed overnight against the same buffer Aliquots were assayed at 30 °C using the method of Brooker[12] Protein determinations were performed according to Lowry *et al*[13] Values represent the average of four experiments
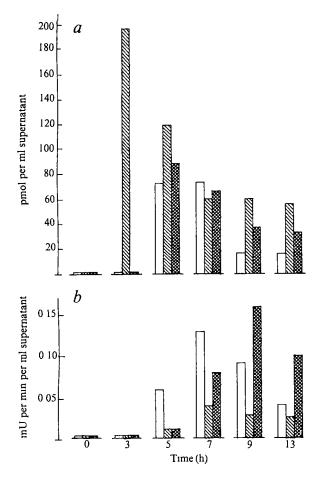
development, we investigated the possible intracellular role of this nucleotide in *D discoideum*

It is generally agreed that abnormal aggregation occurs if amoebae produce an improper chemotactic signal If changes in intracellular cyclic AMP affect processes critical to cell differentiation, however, it is also possible that abnormal aggregation occurs because cells have not been 'induced' to respond properly to a signal A recent study of the effects of various compounds on *D discoideum* revealed that cells treated with either EDTA or progesterone aggregate abnormally[8] In the presence of 10^{-3} M EDTA, a concentration below that of the magnesium ion concentration in the media, cells undergo extensive clumping and very limited streaming This abberant aggregation is completed within the normal period of time, 9 h In contrast, 3×10^{-5} M progesterone causes cells to

remain dissociated and not exhibit any of the morphological changes observed during starvation, even after 18 h incubation When the steroid is removed from the media, cells proceed to aggregate normally Since these two compounds inhibit cell aggregation in markedly different ways, we were interested in determining the level(s) at which they act Here, we report their effects on both intracellular and extracellular cyclic AMP and phosphodiesterase and relate these findings to cell morphology

Figure 1 shows that the intracellular cyclic AMP concentration of amoebae increases by six to eightfold approximately 5 h after transfer on to Petri dishes in non-nutritive media At this time, cells become elongated and establish specific cell–cell contacts typical of the early aggregation phase Similar qualitative changes in cyclic AMP levels in amoebae developing on Millipore filters have been reported[14] The increase in cyclic AMP content is followed by an eight to tenfold induction of phosphodiesterase activity The kinetics of enzymic stimulation correlate well with the decrease in cellular cyclic AMP observed after 7 h Addition of 10^{-3} M EDTA, which enhances the formation of small cell aggregates, produces over a 40-fold stimulation of the cellular cyclic AMP content Even after 11 h, when the cyclic AMP concentration of untreated cells has returned to almost the basal level, that of EDTA-treated cells

Fig 2 Cyclic AMP (a) and phosphodiesterase (b) excreted during starvation by control (open columns), EDTA- (hatched columns) and progesterone-treated (cross-hatched columns) cells Amoebae were incubated as described in the legend to Fig 1 At the indicated times, the media which was aspirated from the cells used for intracellular determinations was either made 1% in TCA and prepared for cyclic AMP assays or dialysed for 5 h against 5 mM Tris, pH 8, 4 mM $MgCl_2$, and then overnight against 50 mM Tris, pH 8, 4 mM $MgCl_2$ The latter samples were then assayed for phosphodiesterase activity
Values represent the average of four experiments

is still greatly elevated This prolonged elevation can be explained by the fact that no increase in phosphodiesterase activity is observed under such conditions Treatment with 3×10^{-5} M progesterone, which results in cells retaining their growth-phase appearance, inhibits both the rise in cyclic AMP and phosphodiesterase activity

The kinetics of cyclic AMP excretion are shown in Fig 2 In the case of untreated amoebae, the concentration of nucleotide in the media increases after 5–6 h of starvation and then decreases This decline is associated with a corresponding increase in an extracellular phosphodiesterase Cells incubated with 10^{-3} M EDTA excrete a large excess of cyclic AMP and do so several hours earlier than control cells During the next 12 h there is a gradual decline in the cyclic AMP level but not a return to that of control cells The small increase in extracellular phosphodiesterase activity seen in EDTA-treated cells is probably responsible for this gradual decrease It therefore seems that EDTA increases the levels of cyclic AMP by inhibiting its intracellular and extracellular degradation Since the extracellular nucleotide is detected early, when cells are incubated with EDTA, it is also possible that synthesis of the nucleotide is stimulated under these conditions Surprisingly, progesterone does not inhibit the appearance of either cyclic AMP or phosphodiesterase in the media, and may, in fact, enhance this process The contrasting effects of progesterone on the internal and external cyclic AMP and phosphodiesterase patterns may be explained by the existence of two systems, only one of which (intracellular) is progesterone sensitive A simpler explanation, however, would be that progesterone stimulates the cellular excretion of both cyclic AMP and phosphodiesterase Other experiments (C K and P B, unpublished) tend to support this model

The variations in the extracellular cyclic nucleotide concentrations are basically unchanged when cells are incubated with steroid Since the changes in extracellular cyclic AMP concentrations occurring in a cell population probably reflect the changes which occur at the individual cell level, this result suggests that the chemotactic signal, thought to orientate cell movement, is unaffected Progesterone probably prevents amoebae from responding to, rather than producing, the chemotactic signal This insensitivity to the chemotactic signal may be directly related to the inability of cells to accumulate cyclic AMP in the presence of steroid The 'inactivated' amoebae therefore tend to remain as isolated cells This interpretation of the intracellular role of cyclic AMP is compatible with the morphological changes seen in the presence of EDTA, that is, the formation of numerous small aggregates EDTA, which greatly increases the cellular levels of cyclic AMP, would produce highly 'activated' amoebae, metabolically prepared to respond to a proper orienting signal Measurements of extracellular cyclic AMP and phosphodiesterase suggest that these amoebae do not produce a proper chemotactic signal They do not stream normally, but instead form small aggregation centres This model predicts that high concentrations of exogenously added cyclic AMP (which would both accummulate within the cells and perturb the extracellular signalling pattern) should mimic the morphological effects seen with EDTA We have found that cells incubated in our conditions with 2×10^{-4} M cyclic AMP form many aggregates without visible streaming (C K and P B, unpublished) Chemotactic assays[4] used as an independent measure of cell differentiation, show also that progesterone-treated cells resemble growth-phase amoebae, whereas EDTA-treated cells behave as differentiated amoebae (C K and P B, unpublished) Obviously, a more extensive analysis must be undertaken to determine the specificity of action of progesterone and EDTA on *D discoideum* These experiments, which include determining their effects on adenyl cyclase, general RNA and protein synthesis, and cellular excretion, are in progress and should help clarify the processes involved in cell aggregation and the levels at which cyclic AMP influences this phenomena

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Carbohydrate binding proteins involved in phagocytosis by *Acanthamoeba*

THE soil sarcodinid *Acanthamoeba castellanii* grows readily in axenic culture[1,2] or in monoxenic conditions with a variety of bacteria as food organisms[3] The reduced growth of this organism in the absence of particulate material[4] suggests that even under axenic conditions the formation of phagocytic vesicles is important in the uptake of nutrients, as in *Tetrahymena*[5] We present evidence that the preferential uptake by *A castellanii* of horse red blood cells over those from other sources[6] is caused by binding of these cells to carbohydrate sensitive sites on the surface of the amoebae, and that these sites may function in the same way in the amoeba's natural habitat

When horse erythrocytes were mixed with logarithmic phase cultures of *A castellanii*, massive mixed-cell agglutination resulted Such a reaction was absent in mixtures of amoebae with human (all ABO groups) or sheep red cells A range of carbohydrates were tested for their ability to inhibit this agglutination (Table 1) The sensitivity of this reaction is similar to that of haemagglutination caused by bacterial type 1 fimbria[7,8] Pretreatment of the horse red cells with concanavalin A (con A) (0 16 mg ml⁻¹) or with phytohaemagglutination (PHA) (Wellcome), did not abolish the mixed cell agglutination, although in the PHA-treated cells simple haemagglutination occurred

The effect of mannose on the preferential uptake of horse red blood cells was investigated by incubating horse and sheep red blood cells with amoebae in a glucose medium and horse red blood cells in a similar medium containing mannose (Fig 1) Clearly the inhibition of the agglutination reaction also inhibits the preferential uptake of horse cells

Carbohydrate-binding proteins have been implicated in many biological activities Rosen *et al*[9,10] have described such proteins on the surface of slime-mould amoebae and have suggested that they are important in the aggregation of these amoebae during plasmodium formation

We suggest, therefore, that *A castellanii* possesses mannose-sensitive carbohydrate binding sites on its surface and that the attachment of particles to these sites facilitates their phagocytosis In contrast Allen *et al*[11] have reported that exogenous lectin (con A) will bind bacteria to the surface of mouse macrophages but this does not lead to phagocytosis, indeed, bound lectin will inhibit the attachment of opsonised bacteria This is not the case in *A castellanii*, in which lectins increase phago-
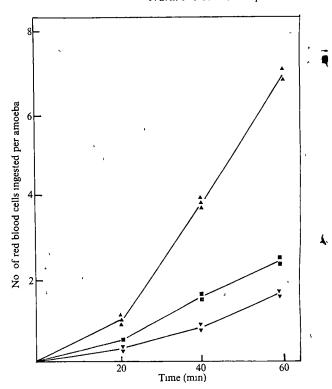
Fig 1 Phagocytosis of horse and sheep red blood cells by *A castellanii* Amoebae from a log phase culture were washed in 0 9% (w/v) NaCl and 1 ml of a suspension put in each of six flasks Medium (7 ml) containing proteose peptone 0 75% (w/v) and yeast extract 0 75% (w/v) were added to each flask, and glucose or mannose was added to a final concentration of 1 5% (w/v) Uptake of horse cells in glucose (▲) and in mannose (▼), is compared with uptake of sheep cells in glucose (■) Uptake was measured spectrophotometrically following hypotonic lysis of uningested red cells and acetic acid extraction of the haemoglobin from the amoebae as described[6]

cytosis[12] The implication of the binding sites which we describe here to the ecology of the amoeba has been investigated by testing a variety of potential food organisms for their ability to form mixed cell aggregates with *A castellanii* and to support monoxenic growth (Table 2) The ability of these particles to attach to the surface of the amoebae, thereby forming aggregates, correlates well with the occurrence of mannose in the

Table 1 Inhibition of mixed cell agglutination of *A castellanii* and horse red blood cells by various carbohydrates

Carbohydrate	Minimum concentration inhibiting mixed cell agglutination
α-Methyl-D-mannoside	3 mM
D-mannose	3 mM
D-fructose	3 mM
Mannan (yeast)*	0 2%
D-glucose	not at 100 mM
D-galactose	not at 100 mM
D-arabinose	not at 100 mM
n-Acetyl glucosamine	not at 100 mM
Sucrose	not at 100 mM
Trehalose	not at 100 mM
L-sorbose	not at 100 mM
Melebiose	not at 100 mM
Inositol	not at 100 mM

Series of double dilutions of the carbohydrates were prepared in a salts solution containing (g l⁻¹) NaCl, 4 0, KCl, 0 2, MgSO₄, 0 05, CaCl₂, 0 07, NaHCO₃, 0 018 and 50 mM phosphate buffer, pH 7 2 A suspension (0 1 ml) of *A castellanii* (10⁸ ml⁻¹) and 0 1 ml of a horse red blood cell suspension (10% v v) were added to each well on a depression plate containing 0 4 ml of the carbohydrate solution The plates were incubated at 30 °C and agglutination results read 20 min later All carbohydrates were obtained from Sigma
*Mannan from *Candida albicans* (a gift from E G V Evans) agglutinated *A castellanii* in the absence of red blood cells

Table 2 Mixed cell agglutination of *A castellanu* and monoxenic growth

Organism particle	Mixed agglutination with *A castellanu*	Supports monoxenic growth
Horse erythrocytes	++++	—
Sheep erythrocytes	—	—
Human erythrocytes	—	—
Saccharomyces cerevisiae CMD 53	++++	+
Saccharomyces carlsbergensis IMI 80178	+++	+
Candida utilis CMD 39	++	+
Shizosaccharomyces pombe	+	+
Pseudomonas aeruginosa PAO 1	—	—
Aerobacter aerogenes NCIB 418	±	+
Bacillus subtilis NCIB 8057	—	—
Stapylococcus aureus CMD 99*	—	±
Polystyrene spheres 9 μm diameter	—	—

Agglutination was tested as described in Table 1 All the organisms were spread on to non-nutrient agar plates and allowed to dry, the plates were inoculated at their centres with drops of log phase culture of *A castellanu*, incubated at 30 °C and examined daily All the agglutination reactions described were sensitive to D-mannose, α-methyl-mannoside and fructose

cell walls of the four yeast species tested Although, under the conditions of our tests, organisms not so bound will support growth of the amoebae, they are present in greater numbers than would be food organisms in the natural environment of *Acanthamoeba* At the lower temperatures and cell populations normally found in the soil, we suggest that phagocytosis would be greatly facilitated by preliminary binding of food organisms to the amoeba It seems likely therefore that yeasts or mannan-containing fungi would form an important source of food for this soil amoeba

Some strains of *Acanthamoeba* will cause menigoencephalitis in experimental animals[13] and humans[14-16] Stevens and Kaufman[17] have already compared binding sites for con A in virulent and non-virulent strains It would therefore be interesting to compare such strains with respect to the lectin-like activity which we describe here

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Visual adaptation in butterflies

THE brilliant glow appearing in the compound eye of butterflies has intrigued investigators of insect eyes since the observations of Exner[1] Miller and Bernard have presented a convincing optical interpretation of the butterfly glow, that the tracheole basal to the rhabdom is modified in such a way as to act as a quarter wavelength interference reflection filter (see refs 2–4) On light adaptation, the eye glow vanishes rapidly[1] Miller and Bernard have conjectured that this is caused by a distal migration of pigment granules in the pigment cells surrounding the crystalline cone[2]

Both the basal reflection theory and the pigment migration hypothesis have been challenged by Swihart et al[5] (highlighted recently[6]) They concluded that the phenomena can be attributed to reflections from corneal processes and photomechanical changes in the retinula cells[5,7], respectively Comparison of our studies on the pupil mechanism of flies[8-10] with similar investigations on the visual adaptation of butterflies[11] suggests that most of the data of Swihart et al[5] strongly support the earlier theories, if these are modified in one important respect—that the distal migration occurs not in the pigment cells but within the retinula cells themselves

This view is presented diagrammatically in Fig 1, showing an ommatidium of a butterfly Since the rhabdom functions as an
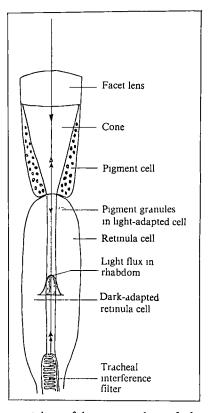
Fig 1 An ommatidium of the compound eye of a butterfly The dioptrical apparatus consists of a transparent facet lens and a cone which are surrounded by heavily pigmented "screening" pigment cells The photoreceptor cells feature a specialised membrane structure, the rhabdomere, which contains the visual molecules Within each ommatidium the rhabdomeres join in a fused rhabdom, an entity which functions as an optical wave guide[11-13] Proximal to the rhabdom an invaginated structure of the tracheal system creates a reflection interference filter So light which enters the ommatidium through facet lens and cone after being propagated by the rhabdom is partially reflected by the basal reflection filter and so can by the same route leave the eye again Obviously a fraction of the light propagated in the rhabdom is absorbed by the visual molecules but owing to the waveguide properties of the rhabdom light is also propagated outside the rhabdom boundary as indicated by the bell-shaped curve, representing schematically one of the possible light distributions Hence pigment granules, existing in the photoreceptor cells as follows from electronmicroscopy (J Tinbergen, unpublished), if localised near the boundary of the rhabdom, can also diminish the light flux, that is, by absorption as well as by scattering By variation of the amount of pigment granules near the rhabdomere the light flux in the butterfly rhabdom can be regulated in an identical way to that occurring in other insects[8-16] In the dark-adapted state (Fig 1, left) the pigment granules are dispersed through the visual cell soma On light adaptation (Fig 1, right) the pigment granules will move towards the rhabdom until an equilibrium situation is reached (see refs 8, 12, 15, 16 and 22)
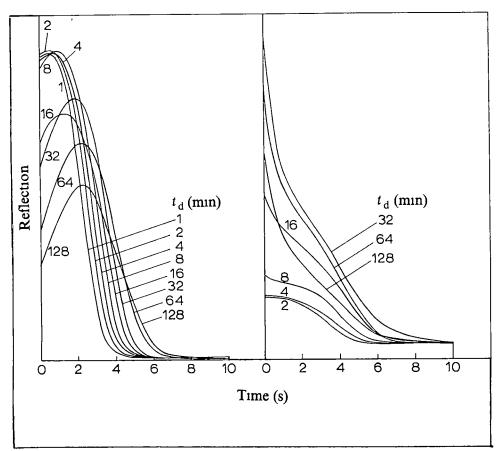
Fig 2 Reflection of the compound eye of the small Tortoiseshell (*Aglais urticae*, Nymphalidae) The curves represent the responses to 10 second pulses of intense light subsequent to a variable dark time t_d, which in turn followed just such a 10 second pulse Irradiation of 584 nm (Fig 2a) and 488 nm (Fig 2b) was applied The initial changes are caused by photochemical events, followed by reflection decreases which are probably due to the migration of absorbing pigment granules towards and against the butterfly rhabdom, thus fulfilling a pupil action The pupil opens during the dark within 0 5-1 min So the initial value of the reflection indicates the state of the visual pigment It proves (Fig 2a) that after prolonged dark adaptation the initial reflection value falls, pointing to dark regeneration of a green rhodopsin This regeneration occurs with a biphasic process, since in the blue the initial reflection first increases but later on decreases again when the dark adaptation time rises above about half an hour (Fig 2b) Possibly a violet absorbing intermediate is involved in the dark regeneration process

optical waveguide the light flux reaching the visual molecules can be controlled by the pigment granules in the photoreceptor cells through regulation of their number near the boundary of the rhabdom (see refs 8-14), a pupil function is in this way fulfilled As a result of the interference reflection filter created by the tracheole basal to the rhabdom, a fraction of the incident light will leave the eye again after a double passage through the rhabdom This fraction of light is the glow visible in the butterfly eye An implication of the outlined conception is that reflection measurements provide knowledge concerning both the visual pigment processes and the pigment migration inside the visual sense cells

The butterfly glow and its disappearance are best seen in the centre of the deep-pseudopupil[1,14], using a vertical-illumination microscope The observations (ref 14, Fig 18) convincingly support the view that the glow is in fact light leaving the rhabdom, and these results disagree completely with the recent proposal[5] that the glow represents reflections arising from the ring of corneal processes surrounding the cone[6,7]

The experimental reflection curves shown in Fig 2 were obtained from the small Tortoiseshell butterfly, *Aglais urticae* (Nymphalidae) A saturating illumination (10 s) of wavelength λ was given after a variable dark-adaptation time t_d, which in turn followed a similar 10 s pulse Figure 2a and b represent the superimposed recordings of the reflected light during light adaptation at λ=584 nm and λ=488 nm, respectively An initial reflection increase (a) or an initial decrease (b), which becomes more prominent as t_d is increased, is followed in both cases by a considerable reflection decrease, the time course of which becomes longer with longer t_d

These observations parallel transmission measurements from blowfly photoreceptors[15] In the latter, intense illumination causes initial transmission changes resulting from photochemical processes in the visual pigment, and subsequently

there is a decrease in transmission which is the consequence of absorbing and scattering pigment granules accumulating near the rhabdomere acting as a light guide[8-16] In the fly, the rate of fall in transmission also slows down with prolonged dark time t_d

Mutatis mutandis, it is reasonable to assign the initial reflection changes in Fig 2 to photochemical processes and to interpret the subsequent fall in reflection as induced by retinula cell pigment granula gathering near the boundary of the butterfly rhabdom, in agreement with the hypothesis stated above Swihart *et al*[5] remark that "should the basal theory be correct, one would imagine that the basal reflections would increase in apparent intensity as the visual pigments were bleached"[5]

Yet, bleaching, implying photolysis of visual pigment, cannot be the correct explanation for the initial events of both Fig 2a and b Rather the transients can be interpreted as representing photochemical conversions of a green-absorbing rhodopsin P525 into a blue-absorbing metarhodopsin M480 and vice versa as has been concluded for the related moths[17,18], similar visual pigment processes have been reported for other invertebrates[19,20]

A photochemical equilibrium between the two pigment states will be established at light adaptation This equilibrium, however, is obviously not maintained during the dark The initial value of the reflection at 584 nm continuously decreases with increasing dark time t_d which implies an increase in absorption at that wavelength, or a regeneration in the dark of the green rhodopsin Although this is not apparent at 584 nm (Fig 2a) the dark process must involve two stages, since at 488 nm (Fig 2b) the initial value of the reflection increases with t_d only during the first half hour of darkness and decreases again after a longer period in the dark

The biphasic dark process can be explained by several

alternatives, such as the blue absorbing metarhodopsin being a quasi-thermostable photoproduct being converted by way of a violet-absorbing intermediate into the native rhodopsin, a blue and a violet metarhodopsin could coexist, each state having its own dark conversion rate, dark conversions of other rhodopsins than that absorbing green could be involved The decision as to which alternative must be ruled out requires a detailed analysis, but the unequivocal establishment of the dark-conversions of the butterfly visual pigment(s) would provide a valuable example of the very scantily known *in vivo* dark processes of invertebrate visual pigments[21]
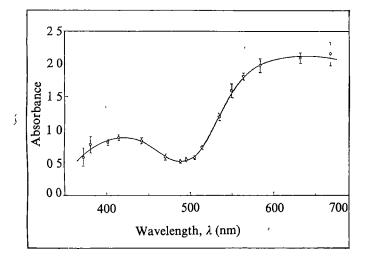
The assumed existence of a green rhodopsin in butterfly eyes is in good agreement with Swihart *et al*[5] that the action spectrum for the extinction of the butterfly glow points to a visual pigment absorbing most strongly at 520–540 nm Hence, the pupil of butterflies could be driven by a force coupled to the receptor potential in the retinula cells in a manner corresponding to the pupil mechanism of flies[8,12,15,16,22]

A further parallel between fly and butterfly pupils may be put forward Illumination of fly photoreceptor cells with intense blue light predominantly converts fly rhodopsin P495 into metarhodopsin M580, resulting in a delayed dark adaptation, which is counteracted by the opposite photopigment conversion[22,23] Furthermore, the pupil of flies absorbs most strongly at just those blue wavelengths which most effectively delay dark adaptation[9,22] We therefore conclude that the fly's pupil not only controls the light flux in the photoreceptor but also its spectral distribution, so as to improve the speed of adaptation

Antagonistic effects of visual pigment conversions on the adaptation of photoreceptor cells have been reported also for the barnacle[20], having a green rhodopsin P532 which is photo-interconvertible with a metarhodopsin M495 In the barnacle it is red light, favouring conversion of rhodopsin to metarhodopsin, which seems to be most effective in delaying dark adaptation of the visual sense cells In the butterfly delayed dark adaptation can also be induced with intense red illumination (D G S, unpublished) Hence, from our knowledge of the pupil of flies we might expect that the butterfly's pupil absorbs extremely in the red to improve the speed of dark adaptation in those photoreceptor cells possessing a green rhodopsin

The absorbance spectrum of the pupil of the small Tortoiseshell butterfly (Fig 3) shows little absorbance in the blue and extreme absorbance in the red, confirming the stated prediction Since we have observed in other butterflies pupil spectra as well as photochemical processes very similar to those reported above, we conclude that, although the visual pigments and the pupil of butterflies and flies seem to have quite different spectral properties, in at least these insect species the pupil may have an

essential function in the speeding up of the dark adaptation after intense illumination It also seems that the butterfly experiments can be satisfactorily interpreted by a combination of the basal reflection theory with the hypothesis of the intracellular pupil

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Fig 3 Absorbance spectrum of the pupil of the small Tortoiseshell *Aglais urticae* The graph represents the difference in absorbance between the 1-min dark-adapted state and the saturating light-adapted state

Evidence for hormonal control of integumentary water loss in cockroaches

The susceptibility of terrestrial insects to desiccation is minimised by extremely effective integumentary waterproofing, which results from the presence of remarkably impermeable epicuticular lipids[1] and, it is suggested, from the properties of the underlying epidermal cells[2] On the basis of rather limited information[3,4] it has, in addition, been suggested that the rate of integumentary water loss may be regulated, the degree of permeability depending on the ambient humidity[2]

We now have evidence that integumentary waterproofing may be hormonally controlled Weight loss is more rapid in decapitated cockroaches than in normal ones or in those in which nervous connection with the brain has been severed (Fig 1) The involvement of a blood-borne factor is also indicated by the significant reduction in apparent water loss after injection of brain extract (Fig 2), or to a lesser extent corpus cardiacum extract, into decapitated individuals

That the accelerated water loss induced by decapitation occurs largely through the integument, and not through the excretory or respiratory systems, is deduced from the following evidence First, anal blocking did not reduce the rate of waterloss in decapitated individuals Second, the activity of thoracic spiracles was reduced following decapitation Third, exposure to 5% CO_2 greatly increased spiracular opening, in both normal and decapitated cockroaches, but did not abolish the more rapid water loss in the latter Finally, spiracular blocking did not significantly reduce water loss in decapitated individuals

The accelerated integumentary water loss induced by
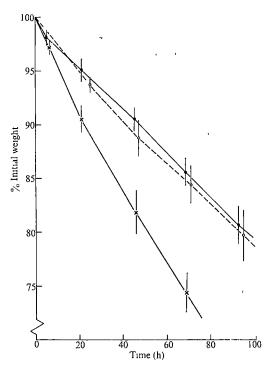
Fig 1 Effects of decapitation and severance of the neck connective on the decline in weight of individuals maintained at 54–55% r h In this, and subsequent diagrams, the symbols indicate the mean of measurements made on ten individuals, the vertical lines indicating the extent of twice the standard error of the mean ●, Normal insects, ○, neck connective severed, ×, headless insects

decapitation could have resulted from nonspecific effects on the permeability of the epidermis, perhaps through an increase of intercellular diffusion resulting from changes in the lateral junctional complexes of epidermal cells (see ref 2) The cation permeability of the cellular layer of the nerve sheath, however, was unaffected by decapitation the extraneuronal potentials, and absence of effects on
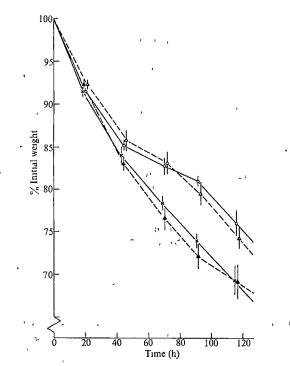
Fig. 2 The effects of injection of brain extract (1 brain + 50 µl saline) on the decline in weight of decapitated insects compared with that of normal and control individuals maintained at 48–50% r h ○, Normal insects, △, headless + brain-extract, ▲, headless + Ringer solution, × headless

the action potentials induced by high-potassium saline were similar to those of normal individuals[5]

It is also possible that the apparent blood-borne factor might act by changing the synthesis and deposition of epicuticular lipids The incorporation of ^{14}C-acetate into epicuticular lipids[6] following injection into the haemolymph, however, was not significantly reduced in decapitated individuals The radioactivity of the lipids washed from the body surface of normal individuals with Triton X and toluene solvent 24 h after injection of 0 5 µCi into the haemolymph was $1,539 \pm 156$ $(n=6)$ d p m compared with $1,436 \pm 260$ $(n=5)$ d p m for decapitated ones

It is, thus, difficult to avoid the conclusion that integumentary permeability may be under neuroendocrine control The apparent involvement of both the brain and the corpus cardiacum suggests a similarity with other systems in which neurosecretory material is synthesised within neuronal cell bodies in the brain and is then transported along specialised axons to the corpus cardiacum (see ref 7)

The prolonged effect of injected brain extract (Fig 2) in reducing water loss suggests that the blood-borne factor is not rapidly metabolised in decapitated individuals A rapid inactivation must, however, be assumed in normal individuals, for injection of brain extract 24 h before the application of a neck ligature produced no appreciable reduction in water loss (Fig 3) This situation seems to be analogous
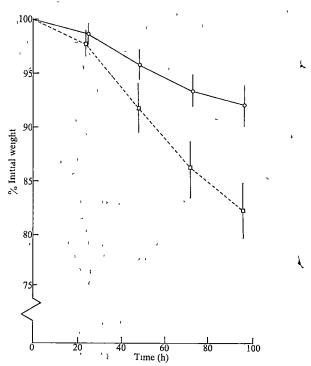
Fig 3 The effect of the application of a neck ligature 24 h after the injection of brain extract (1 brain + 50 µl saline) on the decline in weight at 48–50% r h ○, Normal insects, □, brain extract injected 24 h before application of neck ligature

to that for the diuretic hormone in the tsetse fly, in which it is proposed that an inactivating factor is released into the haemolymph from the thoracic ganglion together with the hormone[8] The persistence of the effects of brain extract in decapitated insects could either mean that the inactivating factor is unstable following extraction, or that it is normally synthesised in the brain only in response to high levels of the waterproofing factor in the haemolymph

Removal of the frontal ganglion or severance of the frontal connectives increases the rate of water loss from cockroaches and it is suggested that the frontal ganglion receives an input from osmoreceptors[9] The frontal ganglion could also be involved in mediating the release of water-

proofing factor from the brain by way of the corpus cardiacum

Little is known concerning the mode of action of the postulated blood-borne factor It has been shown, however, that decapitation has little effect on the electrical resistance of the integument[10] The resistance for normal pronotal integument averaged $3,571 \pm 1,324\,\Omega\,cm^2$ as compared with $3,661 \pm 1,020\,\Omega\,cm^2$ measured in decapitated individuals The observations would accord with the idea, for example, that transpiration is increased by alterations in an oriented epicuticular lipid layer[1] which may be insufficient to affect permeability to inorganic cations and thus the flow of current across the integument

The physiological role of a neuroendocrine control of integumentary transpiration remains to be elucidated It would be an obvious advantage for insects to be maximally waterproof in dry conditions It is conceivable, however, to cite a single example, that under conditions of excessive water intake (such as might occur with a liquid or semi-liquid diet of low nitrogen content) it could be physiologically advantageous to increase the rate of integumentary transpiration Alternatively, it could be that this apparent neuroendocrine control ensures that the organisation of the epicuticular lipids is maintained so as to ensure maximal waterproofing An example of such a maintenance function could be the controlled release of the antioxidant, protocatechuic acid, which is necessary to prevent the degradation and polymerisation of epicuticular lipids[11]

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Anaesthetisation of prefrontal cortex and response to noxious stimulation

REPORTS indicate that responses to pain may be modified by damage to the frontal lobes (see refs 1 and 2) Nevertheless, neither the nature of the change nor the degree to which any effect of damage is localised anatomically has been firmly established I have systematically examined the function of the prefrontal cortex in response to an aversive stimulus in the rat Small quantities of a short-acting anaesthetic, procaine hydrochloride, were injected to block directly the neural function of specific areas within the prefrontal cortex The prefrontal cortex in the rat occupies both a lateral region, the dorsal bank of the rhinal sulcus, and a medial region, the pregenual medial wall of the hemisphere[3] In the rat, the prefrontal cortex is involved in brain-stimulation reward[4] I suggest that, in addition, the prefrontal cortex acts to limit the response to painful stimulation, and that this anti-nociceptive function is localised in or near the sulcal region

Following the bilateral injection of 1 0 μl 5% procaine hydrochloride (see legend to Fig 1), the preinjection jump threshold of 0.86 ± 0.22 mA (mean $\pm$ s d) was reduced to 0.77 ± 0.19 mA ($t=6.2$, $P<0.001$), and after bilateral injection of 2 μl, it was further reduced to 0.73 ± 0.21 mA ($t=6.9$, $P<0.001$) Every animal exhibited a reduction in jump threshold following both the 1 0 and 2 0 μl bilateral injections The
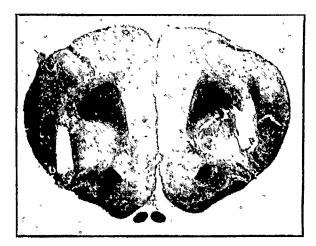
Fig 1 An example of bilateral cannula tracks with the tips in the sulcal prefrontal cortex For the cannula implantation, level-head stereotaxic coordinates[6] were used Adult male, hooded rats (300 g) were cannulated and injected as described elsewhere[4] Each rat was implanted bilaterally with stainless steel guide cannulae, fitted with an obturator The latter was removed and replaced with an injection cannula before an injection experiment Ten rats were implanted bilaterally with cannulae in the sulcal prefrontal cortex The stereotaxic coordinates of each cannula tip were 2 3 mm anterior to the bregma, 3 5 mm lateral to the sagittal sinus, and 4 0 mm beneath the dorsal surface of the brain (+2 3 3 5 4 0 mm down) Either 5% procaine hydrochloride in 0 9% saline or saline alone was injected bilaterally Responses to aversive footshock were determined using a 'flinch–jump' test[5] Each rat received six series of ten shocks per series, in which scrambled electrical shocks (0 5 s duration) were delivered at 15 s intervals to the floor of the test box The shock intensities in each series were ten consecutive values from the range 0 13 to 1 60 mA, with 60 s between each series Shocks were delivered in ascending and descending order on alternate series The rat's response to each shock was recorded as either no response, flinch (startle, including forepaw-shaking), or jump (both rear paws leave floor) The jump response alone seems to be related to specifically painful stimulation, since morphine greatly increases the jump threshold without affecting the flinch threshold[5] The flinch and jump thresholds were noted for each series, and the mean values over the six series calculated The rat was then removed, injected bilaterally with 1 0 μl 5% procaine hydrochloride, and immediately returned to the test box Six more shock series were run to determine postinjection flinch and jump thresholds After 30 min, the effects of the procaine having dissipated, the rat was reinjected with 2 0 μl 5% procaine hydrochloride, and a second series of six postinjection series were run The same procedure was repeated no sooner than 48 h afterwards, to determine the effects of the injection of 0 9% saline For statistical analysis, the threshold results obtained for the pre- and postinjection series were compared using a correlated t test (two-tailed)

flinch threshold, in contrast, remained unchanged The pre-injection flinch threshold was 0.19 ± 0.05 mA, after bilateral injection of 1 0 μl procaine hydrochloride 0.18 ± 0.03 mA, and after 2 0 μl bilaterally 0.18 ± 0.03 mA (non-significant differences) In the saline control experiments, the preinjection jump threshold of 0.86 ± 0.22 mA did not differ significantly from the threshold value 0.87 ± 0.22 mA after the injection of 2 0 μl saline bilaterally The lowered jump threshold which followed bilateral anaesthetisation of the sulcal prefrontal cortex did not result from a nonspecific reduction in response thresholds, since first, the flinch thresholds remained unchanged, and secondly, such anaesthetisation has been shown to increase self-stimulation thresholds[4] Thirdly, while the anaesthetic was effective, the general behaviour of the animals seemed to be unaffected (see also ref 4) Bilateral procaine injections seemed to be necessary since, in subsequent work, no effect of unilateral procaine injection on the jump threshold was observed An example of histology which shows that the tips of the cannulae were placed bilaterally in the sulcal region is shown in Fig 1

Additional experiments were run to determine the degree

of localisation of the effect of bilateral anaesthetisation on the jump response to footshock Four rats were implanted with bilateral guide cannulae aimed at the medial prefrontal cortex (+2 3 2 0 2 4 mm down, angled 28° towards the midline) and three rats with bilateral cannulae aimed 1 0 mm behind the effective sulcal placement The animals were tested as above Bilateral injections of 1 0–2 0 μl procaine hydrochloride, or 0 9% saline, had no effect on either the jump or the flinch thresholds in both groups of animals This series of experiments demonstrates conclusively that bilateral anaesthetisation of a region in or near the sulcal prefrontal cortex lowers the threshold of the jump response elicited by footshock The jump response, in contrast to the flinch response, seems to be elicited by painful consequences of footshock[5] A region specifically in or near the sulcal prefrontal cortex seems to be implicated, since procaine injected into either the medial prefrontal cortex or a site 1 0 mm behind the effective site had no effect on the jump threshold The conclusion that, in the intact rat, a region in or near the sulcal prefrontal cortex functions to limit the response to painful footshock, is supported by the results of these experiments Also of interest was whether the prefrontal cortex is a site of action of the potent analgesic drug, morphine Since bilateral injections of morphine sulphate (4% solution) into either the sulcal or the medial prefrontal cortex do not affect the jump threshold (S J C , unpublished), it would seem unlikely that morphine analgesia[5] involves any action within the prefrontal cortex

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Pre- and postsynaptic components in effect of drugs with α adrenoceptor affinity

ACTIVATION of α adrenoceptors on noradrenergic nerve endings is thought to inhibit the release of noradrenaline by nerve impulses[1,2] The existence of a presynaptic receptor system introduces a new concept to the pharmacology of drugs with affinity for α adrenoceptors Only when the presynaptic nerve does not secrete, as for example in the absence of impulses, may one assume *a priori* that the observed postsynaptic effects of these drugs result from their interaction with postsynaptic receptors When action potentials arrive at the nerve endings, noradrenaline is released and keeps the postsynaptic receptors in a certain degree of excitation Drugs which activate α adrenoceptors may enhance this excitation by their postsynaptic effects, or reduce it by their presynaptic actions Conversely, α adrenolytic drugs may diminish the excitation by postsynaptic blockade, or enhance it by facilitation of release If the postsynaptic receptors are of the β type, the prevailing action is presynaptic, thus, drugs which activate α adrenoceptors reduce[3–5], whereas, less regularly, α adrenolytic drugs increase[6] cardiac responses to sympathetic nerve stimulation If the postsynaptic receptors are of the α type, the prevailing effect of α adrenolytic drugs is postsynaptic blockade[7], we report here that the effects of drugs which activate α adrenoceptors are diverse

The experiments were performed on rabbit main pulmonary arteries, in which postsynaptic α adrenoceptors mediate

vasoconstriction Artery strips were superfused with Krebs–Henseleit solution containing propranolol, cocaine and corticosterone to block β adrenoceptors and the neuronal and extraneuronal uptake of noradrenaline, respectively Actions on postsynaptic α adrenoceptors were studied by determining dose–response curves in the absence of nerve impulses For the separate study of presynaptic actions, arteries were preincubated with ³H-noradrenaline, and drug effects on the overflow of tritium evoked by transmural electrical stimulation were measured The mode of stimulation used selectively excites the adrenergic nerves of the arteries, the stimulation-induced overflow of tritium reflects the stimulation-induced secretion of noradrenaline from nerve terminals[8,9] To study the influence on postsynaptic cells during active neurotransmission, frequency–response curves were elicited before and after the addition of the drug

Isolated pre- and postsynaptic actions of oxymetazoline and phenylephrine are shown in Fig 1 Oxymetazoline preferentially activated presynaptic α adrenoceptors and thus inhibited release, whereas phenylephrine preferentially activated postsynaptic receptors and thus induced contraction Methoxamine behaved like phenylephrine, clonidine has been shown to behave like oxymetazoline[8] The differences in the relative potencies of these drugs may reflect structural differences in pre- and postsynaptic binding sites

Stepwise increases in the frequency of transmural stimulation induced stepwise contraction, maximal at 16 Hz Figure 2 illustrates the influence of drugs which activate α adrenoceptors at low concentrations which, in the absence of impulse flow, caused contractions ranging between 1 0% and 3 7% of the maximal response to noradrenaline As would be predicted from their relative pre- and postsynaptic potencies, oxymetazoline and clonidine reduced, whereas phenylephrine and methoxamine enhanced neurogenic vasoconstriction The effect of α adrenoceptor-activating drugs in inhibiting noradrenaline release, declines as the frequency of stimulation is increased[3,5] Accordingly, clonidine and oxymetaxoline inhibited smooth muscle responses to stimulation at low, but not at high frequency The
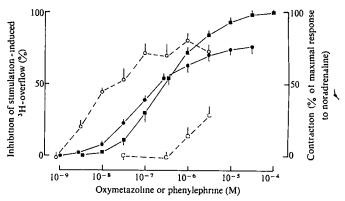
Fig. 1 Pre- and postsynaptic actions of oxymetazoline (○, ●) and phenylephrine (□,■) in the rabbit pulmonary artery Left ordinate, presynaptic action (○,□) Arteries were preincubated with 1 5 × 10⁻⁸ M (—)-³H-noradrenaline for 1 h and then superfused with fresh medium containing 3 × 10⁻⁵ M cocaine, 4 × 10⁻⁵ M corticosterone and 4 × 10⁻⁶ M propranolol Each strip was stimulated five times (S₁–S₅) for 3 min with pulses of 0 3 ms duration and 150 mA current strength at 2 Hz Stimulation periods started after 126, 144, 162, 180 and 198 min of superfusion Oxymetazoline or phenylephrine were added 12 min before S₄ Phenylephrine concentrations exceeding 3 × 10⁻⁶ M could not be tested, since they accelerated basal tritium outflow In control experiments, the ratio between the overflow of tritium evoked by S₄ and that evoked by S₃ was 0 97 ± 0 03 (n = 11) The ordinate indicates percentage decreases of the ratio caused by the drugs Each point is the mean ± s e m of four to eight experiments Right ordinate, postsynaptic action (●,■) Two dose–response curves were determined on each strip, first a curve for noradrenaline, then a curve for oxymetazoline or phenylephrine Results are corrected for the increase of contractions with time[8] Values are the means ± s e m of five dose–response curves for oxymetazoline and phenylephrine

inhibition was observed with 10^{-8} — 10^{-7} M clonidine[8] and 10^{-9} — 3×10^{-9} M oxymetazoline, at higher concentrations, postsynaptic receptor activation prevailed and vasoconstriction was enhanced In contrast, phenylephrine and methoxamine uniformly enhanced vasoconstriction in response to low frequency stimulation at concentrations from 3×10^{-9} — 3×10^{-8} M and 3×10^{-8} — 3×10^{-7} M, respectively

In vivo, action potentials continuously arrive at nerve endings Our results demonstrate that in this situation a presynaptic component significantly contributes to the overall postsynaptic effect of some drugs activating α adrenoceptors, even if postsynaptic receptors are of the α type Selective pre- and postsynaptic receptor activation may also explain the diverse effects of α sympathomimetic drugs on the response of vas deferens to stimulation of its motor nerves [5,10-12] Detailed analyses of preand postsynaptic components may lead to a more complete understanding of the pharmacology of drugs that interact with α adrenoceptors

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Affinity partitioning of acetylcholine receptor enriched membranes and their purification

AFFINITY chromatography, which uses a specific ligand coupled to a solid matrix to adsorb selectively a macromolecule, has been used extensively in the purification of soluble proteins[1] Although the purification of cells or cellular membrane fragments containing surface receptors has been attempted with this procedure[2-15], the approach has been less successful because of difficulties in eluting bound particulate substances from the solid matrix Recently, we described a method called affinity partitioning[16] for separating soluble proteins in aqueous polymer two-phase systems[17] by adding a polymer-ligand with a relatively high affinity for a binding site on the protein to be purified, and a solubility preference for one of the phases Since cells and cell fragments[18,19] can be partitioned and recovered from aqueous polymer two-phase systems, it seemed possible that the principle of affinity partitioning could be used in their fractionation With the absence of a solid matrix, problems of recovery would be obviated We describe here the use of polymer coupled to a ligand that binds to the nicotinic cholinergic receptor site to purify cholinergic receptor enriched membranes derived from electroplax of *Torpedo californica*

This membrane system was used to evaluate application of affinity partitioning to particulates because of the following favourable characteristics first, the membranes contain unusually high receptor densities (up to $30,000\ \mu m^{-1}$)[20,21], second, there is detailed knowledge of the ligand specificity and molecular properties of the binding site[22], third, differential and sucrose density gradient centrifugation have shown[23] that discrete membrane fragments enriched in nicotinic cholinergic

Concentration of poly (EO) derivative (M)
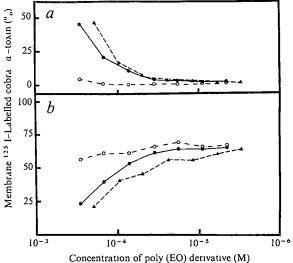
Fig 1 Distribution of membrane-bound ^{125}I-labelled α-toxin in presence of various concentrations of MA-poly(EO) (○), TMA-poly(EO) (●) and PTMA-poly(EO) (▲) The final phase system contained (per kg) 4 32% (w/w) Dextran T-500, 3 59% (w/w) poly(EO) 6,000, 1 25 mmol sodium phosphate, pH 7 4, 4 75 mmol NaCl Unsubstituted poly(EO) was replaced with an equal amount of poly(EO) derivative to give the indicated concentrations of terminal ligands Substitution grades of PTMA-poly(EO), MA-poly(EO) and TMA-poly(EO) were calculated from elemental analysis to be 66%, 96% and 103%, respectively, where 100% substitution grade is two terminal molecules of ligand per linear poly(EO) molecule We express the molarity of polymer-ligand as the ligand molarity, were it free in solution Sufficient iodinated toxin to occupy approximately 7 3% of the α-toxin binding sites was added to a membrane fraction purified by sucrose density gradient centrifugation[23] Phase systems containing membrane-bound ^{125}I-labelled α-toxin were mixed and placed on ice for 15 min and then centrifuged at 500g for 15 min at 5 5 °C Aliquots of top (*a*) and bottom (*b*) phases were collected and counted in a Nuclear Chicago gamma-counter

Table 1 Effect of bisquaternary methonium compounds on toxin binding and affinity partitioning

	Concentration (M) that inhibits initial rate of toxin binding by 50%	Concentration (M) that inhibits PTMA-poly(EO) effect by 50%
Decamethonium	3×10^{-8}	2×10^{-7}
Hexamethonium	1×10^{-6}	3×10^{-6}
Trimethonium	7×10^{-5}	$> 5 \times 10^{-4}$

Initial rate of ^{125}I-labelled *Naja naja siamensis* α-toxin binding was determined by a modification of the method of Schmidt and Raftery[26] Reaction mixtures contained 4×10^{-8} M acetylcholine receptor and fourfold molar excess of ^{125}I-labelled *Naja naja siamensis* α-toxin (5–10 Ci mmol^{-1}) in 10 mM phosphate, pH 7 4 The reaction rate approximated pseudo first-order kinetics During incubation in the absence of inhibitory ligand the reaction progressed to 30–40% of completion and initial rates were calculated assuming a first order approach to equilibrium The concentrations of bis-quaternary methonium compounds required to inhibit the affinity partitioning of membrane-bound ^{125}I-labelled α-toxin in the top phase was determined using a system like that in Fig 1 with 1 85 × 10^{-4} M PTMA-poly(EO)

receptors, or Na$^+$-K$^+$-stimulated adenosine triphosphatase, or membrane-bound acetylcholinesterase can be fractionated from homogenates of the electric organ of *Torpedo californica*

A standard two-phase system containing dextran and polyethylene oxide was used The polymer-ligand, α,ω-*bis*-4-trimethylammonium (phenylamino) polyethylene oxide [PTMA-poly(EO)] was synthesised from (4-aminophenyl) trimethylammonium hydrochloride iodide (Eastman) and α,ω-dibromopolyethylene oxide by a method based on that described by Johansson[24] The quaternary ligand we used has been coupled to Sepharose through a side arm and used for affinity chromatographic purification of acetylcholinesterase and detergent-solubilised receptor[25] Since this compound like most others known to react with the cholinergic receptor is charged, other cationic ligand-polymer conjugates with anticipated differences in receptor affinity, α,ω-*bis*trimethylammonium polyethylene oxide [TMA-poly(EO)] and α,ω-*bis*methylamino polyethylene oxide [MA-poly(EO)] were examined for comparative purposes At the same level of substitution, the conjugates would impart the same charge to the phase system

Initial studies were conducted with electroplax membranes partially purified by density gradient procedures[23] Cholinergic receptor concentrations in the individual phases were monitored with ^{125}I-labelled *Naja naja siamensis* α-toxin (5–10 Ci per mmol) using the assay procedure of Schmidt and Raftery[26] This assay, and a simpler monitoring procedure that consists

Table 2 Specific activity of fractions from single extractions in phase system containing PTMA-poly(EO)

	ATPase*	AChE†	Toxin binding‡
Crude pellet	62 8	35	353
Bottom phase	26 4	20	201
Top phase	2 5	7	2,499

The phase system used in this extraction contained (per kg) 4 32% (w/w) Dextran T-500, 3 59% (w/w) poly(EO) 6,000, 1 85 × 10^{-4} M PTMA-poly(EO), 24 75 mmol NaCl, 4 75 mmol sodium phosphate, pH 7 4 A particulate fraction was obtained from a homogenate of electroplax by differential centrifugation[18] This fraction, containing 15 6 mg protein was resuspended in 2 ml of 1 mM phosphate buffer, pH 7 4, and added to the phase system to give the above concentrations in 40 g of total system The mixture was placed on ice, mixed by repeatedly inverting the tube, and then added to a 60-ml syringe fitted with a valve The syringe assembly was centrifuged at 1,000 r p m for 30 min at 5 5 °C in a GLC-1 centrifuge (Sorvall) The top phase was collected directly and the bottom phase was collected through the valve The top and bottom phases were diluted with distilled water and centrifuged for 3 h at 35,000 r p m in a type 35 rotor (Beckman Instruments) Of the protein added to the phase system, 6 63 mg was recovered in bottom phase and 0 51 mg was recovered in top phase No determination was made of the constituents in the interphase
*μmol of ATP hydrolysed per h per mg protein[28]
†μmol of acetylcholine hydrolysed per min per mg protein[29]
‡pmol of ^{125}I-labelled *Naja naja siamensis* toxin bound per mg protein[26]

of addition of toxin at concentrations less than 7 5% of site saturation before phase separation, yielded similar values for the partitioning of receptor between the two phases

In a conventional aqueous polymer two-phase system (Fig 1) less than 1% of membranes containing the cholinergic receptor was found in the polyethylene-oxide-rich top phase and about 65% was in the dextran-rich bottom phase The remaining material was presumably localised to the interphase When PTMA-poly(EO) was added there was a striking increase in the concentration of the membranes containing receptor in the top phase and a commensurate reduction in the bottom phase (Fig 1) Addition of TMA-poly(EO) produced similar results although this polymer-ligand was somewhat less potent than PTMA-poly(EO) in this and other studies (Fig 1) When MA-poly(EO) was used there was only a slight effect on the partitioning of the cholinergic-receptor-containing membranes even when relatively high concentrations were used (Fig 1) Since MA-poly(EO) is a secondary amine which is about 99% charged at the pH used in these studies (7 4), the addition of a cationic polymer-ligand, which might be expected to alter the E M F between phases[27], is not responsible for the partitioning effect that we observed

To examine further whether the effect of PTMA-poly(EO) on the partitioning of membranes containing the cholinergic receptor was the result of specific binding to the cholinergic receptor site, we studied the effects of three bisquaternary methonium compounds with differing affinities for the cholinergic receptor The affinity of these compounds for the cholinergic receptor was measured from inhibition of the initial rate of binding of ^{125}I-labelled α-toxin to membrane-bound receptor (Table 1) As expected, decamethonium was more potent than hexamethonium, which in turn was more potent than trimethonium Addition of each of these compounds to the aqueous polymer two-phase system in the presence of PTMA-poly(EO) inhibited the affinity partitioning (Table 1) with an order of potency related to their affinity for a ligand-binding site on the receptor We also determined the effect of progressive saturation of the cholinergic receptor with increasing concentrations of α-toxin (unlabelled) on the affinity partitioning effect of PTMA-poly(EO) Progressive addition of increasing concentrations of α-toxin antagonised the affinity partitioning effect of PTMA-poly(EO) in direct proportion to the saturation of toxin binding sites Binding of toxin is known to compete with ligands behaving as agonists and antagonists for the nicotinic receptor[22]

Having established that affinity partitioning could be applied with partially purified membranes, we investigated whether it could be used to purify membranes containing the cholinergic receptor from a crude mixture of membranes obtained from a homogenate of electroplax When this mixture was added to a system containing 1 85 × 10^{-4} M PTMA-poly(EO), there was about a sevenfold increase in the specific activity of toxin binding in the membranes that partitioned to the top phase, as compared with the crude particulate fraction (Table 2) About 25% of the toxin binding activity was recovered in the top phase The specific activity of ATPase in the membranes of the top phase was less than 5% of that of the crude particulate fraction and the specific activity of acetylcholinesterase in the membranes of the top phase was about 20% of that of the starting extract It should be emphasised that all these activities are membrane bound since they were collected by centrifugation after the partitioning was completed Partitioning of these membranes in an identical aqueous polymer two-phase system in the absence of PTMA-poly(EO) showed that the top phase contained less than 2% of the toxin binding activity, acetylcholinesterase or ATPase added to the system

The purification achieved with a single partitioning step is comparable with that achieved by sucrose density gradient fractionation[23], since about 25% of the total protein in the membranes found in the top phase after affinity partitioning was found to be cholinergic receptor protein Experiments in progress suggest that even greater purification can be achieved

either by sequential affinity partitioning steps or by combining the sucrose gradient centrifugation purification procedure with affinity partitioning

These results suggest that a similar approach might be applied to the purification of cell membranes or even intact cells that contain sufficient concentrations of specific receptors Preparative scale purifications should be possible since large volumes of two-phase systems can be handled conveniently

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Uptake of noradrenaline by an adrenergic clone of neuroblastoma cells

THERE are specialised transport systems mediating noradrenaline (NA) uptake from the extracellular fluid in various types of neurones in peripheral and central noradrenergic nerve terminals

A specific and saturable high affinity uptake for the NA precursor tyrosine has been demonstrated in the adrenergic neuroblastoma clone NIE-115 (ref 1) Another adrenergic neuroblastoma clone, M1 (ref 2) possesses a transport system for tyrosine with similar characteristics Cells of the M1 clone can synthesise ³H-NA from ³H-tyrosine, the ³H-NA formed is found not only in the cells, but also in the medium where it attains a higher specific activity than in the cell interior (J Z, unpublished) Thus a system may be operating which permits the passage of NA across the membrane Here, we present data

which demonstrate the existence of an uptake for NA itself in the adrenergic neuroblastoma clone M1

Cells of the adrenergic clone M1 isolated from C-1300 neuroblastoma were cultivated in Dulbecco's modified Eagle's MEM with foetal calf serum under an atmosphere of air-CO_2 at 37 °C Assays were performed in a phosphate-buffered saline medium (PBS) containing labelled NA (Fig 1) The effect of amphetamine, desipramine, imipramine and ouabain on NA uptake was tested in the same medium

When the initial velocities of L-³H-NA uptake by the neuroblastoma cells were plotted[3] against various amine concentrations, the presence of two components was revealed (Fig 1) One component operating at low NA concentrations has an apparent K_{m1} of 0 14 µM This value for the high affinity transport of NA is similar to that reported for the isolated heart[4] and synaptosomal preparations[5-7] Another component which corresponds to a low affinity system has an apparent K_{m2} of 2×10^{-5} M This K_{m2} value was determined by incubation in NA concentrations $(3 \times 10^{-7}–5 \times 10^{-4}$ M) higher than indicated in Fig 1 It is possible that this system is related to a non-saturable component Free diffusion, however, cannot totally account for this NA influx, in view of the data in Table 1 which indicate that the low-affinity system is partially energy dependent

The uptake of NA was inhibited when a metabolic poison 2,4-dinitrophenol (DNP) was added to the incubation medium in high concentrations (2 mM) 30 min before the addition of
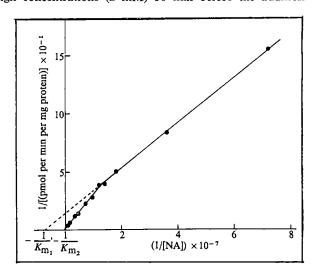
Fig. 1 Double reciprocal plot of L-³H-NA uptake into adrenergic clone M1 against NA concentrations Cells were grown to confluency (4 d) in Petri dishes in Dulbecco's modified Eagle's MEM with 10% foetal calf serum under an atmosphere of 5% CO_2 in air at 37 °C Assays were performed at 37 °C in 5 ml of phosphate buffered saline (PBS) containing 137 mM NaCl, 2 6 mM KCl, 0 7 mM $CaCl_2$, 0 5 mM $MgCl_2$, 3 2 mM Na_2HPO_4, 1 4 mM KH_2PO_4, 10 mM glucose The pH was adjusted to 7 3 (at 37 °C) by addition of NaOH The osmolarity was 305 mosmol l^{-1} This medium was not found to have any detrimental effect on cell viability as judged by Trepan blue colouration Ten minutes after replacement of the growth medium by PBS, the reaction was started by adding L-³H-NA (the Radiochemical Centre, Amersham, specific activity 7 2 Ci $mmol^{-1}$) Substrate was present in the reaction mixture at concentrations from $1 4 \times 10^{-8}$ to 10^{-6} M The length of incubation was 2 min, preliminary experiments had shown that the uptake was linear for this time period Reactions were terminated by removal of the incubation medium followed by four washes with NaCl 9‰ at 37 °C Cells were quantitatively harvested with 1 ml and one wash of 0 8 ml of distilled water Then, 0 2 ml NaOH N were added An aliquot (1 5 ml) of this solution was counted in the presence of 10 ml scintillation mixture (Monophase 40, Packard) in an Inter-technique SL-30 liquid scintillation spectrometer Protein was determined by the method of Lowry *et al* [18] using bovine serum albumin as standard Each point is the average of four determinations Velocities are expressed in 10^{-12} mol ³H-NA per min per mg protein The following kinetic constants were determined using the method of Wilkinson[19] High affinity transport apparent $K_{m1} = 0 14$ µM, apparent $V_{max1} = 0 07$ pmol per min per mg protein Low affinity transport apparent $K_{m2} = 2 10^{-5}$ M, apparent $V_{max2} = 5 4$ pmol per min per mg protein

Table 1 Effect of various drugs and Na^+ deprivation on NA uptake

	(NA) 1 4 × 10^{-8}M		(NA) 2 × 10^{-5} M	
Control	100 ± 18	(12)	100 ± 17	(4)
DNP 2 mM	73 ± 10*	(4)	69 ± 8*	(4)
4 °C	36 ± 7‡	(4)	17 ± 7‡	(4)
Sucrose	53 ± 2‡	(4)	72 ± 2*	(3)
Ouabain 10^{-4} M	58 ± 2‡	(4)	101 ± 15	(4)
Amphetamine 5 10^{-5} M	75 ± 4*	(5)	113 ± 23	(4)
Desipramine 10^{-5} M	61 ± 5†	(3)	101 ± 27	(4)
Imipramine 10^{-5} M	62 ± 6†	(4)	126 ± 30	(4)

Cells were grown and assayed as described in Fig 1 ^{3}H-NA was added after a 15-min preincubation with the drugs, except for DNP (30 min) and the reaction was stopped after 5 min Each determination was repeated as indicated Data are expressed as percentages of control uptake ± s d, determined as d p m of ^{3}H per mg protein in the dish When Na^+ was omitted, sucrose was added in the same osmolarity Control values (100%) for uptake (d p m of ^{3}H per mg protein ± s d) were 3,884 ± 706 and 1,818 ± 306 for NA concentration of 1 4 × 10^{-8} M and 2 × 10^{-5} M respectively Significance, as judged by Student's t-test is indicated as follows
$* P < 0 05$
$† P < 0 01$
$‡ P < 0 001$

labelled substrate Those conditions have been reported to inhibit NA uptake into synaptosomal preparations[8]

In addition of being energy-dependent the accumulation of NA was found to be temperature-sensitive At 4 °C, both components of the NA uptake were greatly reduced The high affinity process seemed to be dependent on the presence of Na^+, since it was greatly reduced by replacing the NaCl in the incubation medium by sucrose of the same osmolarity

In similar conditions, addition of ouabain (10^{-4} M) inhibited the high affinity uptake only The low affinity process was less markedly sodium-dependent and was found to be not ouabain-sensitive These data are in good agreement with those of Iversen[9]

Potent inhibitors of catecholamine uptake such as imipramine, desipramine[4,10,11] or amphetamine[12,13] all inhibited the uptake of NA, but only that by the high affinity process (Table 1) Amphetamine was the least effective drug The inhibition, however, was less marked than that described by Baldessarini and Vogt[7] for rat brain homogenates This relatively weak inhibition cannot be explained by the destruction of NA in the incubation medium since in separate experiments, we have observed that after 5 min of incubation, it remains at least 80% of the initial NA level Thus our observations are probably the result of a difference between the neuroblastoma cell membrane and the cell membranes studied by other authors[4,10-13]

The uptake of NA in the adrenergic clone M1 of C-1300 neuroblastoma seems to have similar properties as the neuronal uptake studied by Iversen[4,9] First, a saturable high affinity transport system was found which was partially energy dependent and temperature sensitive This uptake seems to require Na^+ and to be inhibited by ouabain, amphetamine, imipramine and desipramine Second, the presence of a saturable low affinity transport system was demonstrated This low affinity uptake system was also partially energy dependent and temperature sensitive, but was not inhibited by the drugs tested

In many respects, neuroblastoma cells grown in culture may be used as model systems for nerve cells[14-17] We have shown that this applies also for NA uptake into an adrenergic clone The similarities between NA uptake operating in the cultured cells and those shown to exist in other systems are especially noteworthy Most studies with NA uptake processes have been done with nerve ending preparations or with tissues containing adrenergic nerve terminals, whereas cultivated neuroblastoma cells are devoid of synapses When moreover nerve terminals are present, the investigation of transmitter uptake by the neuronal membrane is complicated by the presence of a second uptake into the storage granules Adrenergic neuroblastoma clones may therefore represent a useful system for the study of catecholamine transport processes across the neuronal membrane

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Mode of action of an anti-inflammatory fraction from normal human plasma

A FRACTION isolated from normal human plasma[1], contains a substance of low molecular weight (< 500) which shows anti-inflammatory activity in a number of animal models, including paw oedema tests in the mouse and rat[1,2], adjuvant arthritis in the rat[3] and Arthus reactions in the rat and rabbit[4] Its activity in the carrageenin-induced paw oedema test in the rat does not involve an interference with either the release or action of chemical mediators of inflammation such as histamine, 5-hydroxytryptamine, kinins or prostaglandins[5,6] The fraction is not active in inflammatory reactions in which these mediators play the more prominent role, for example, passive cutaneous anaphylaxis and the extravasation of plasma protein elicited by intradermal challenge with the mediators[4], but shows marked and reproducible anti-inflammatory activity in situations in which the emigration of circulating leukocytes is a major factor, for example, Arthus reactions and the accumulation of polymorphonuclear and mononuclear cells into pleural and other inflammatory exudates[7] The plasma fraction causes a reduction of up to 90% in the migration of leukocytes into the exudates found in porous inert sponges implanted subdermally in the rat[7]

An important group of substances thought to induce the directed migration of leukocytes from the blood through the tissue extravascular spaces are the chemotactic factors derived from the complement system[8] Although chemotaxis is an extremely difficult phenomenon to prove by direct observation *in vivo* the circumstantial evidence for it is overwhelming[9] One example is the inhibition of migration of leukocytes into implanted sponge exudates in the rat when the circulating complement levels are depleted by treatment with purified cobra venom[10] The plasma fraction does not act by depleting serum complement levels *in vivo* since it has no effect on the circulating titre of total haemolytic complement in the rat Neither does it prevent the activation of the classical or alternate pathways of complement in fresh guinea pig serum by either antigen–antibody complexes, heat-aggregated γ-globulin or zymosan, as assessed by measuring the residual total haemolytic titre[7]

The preparations of the plasma fraction used were fraction II (ref 1) further purified by absorption on Sephadex A25 ion-exchange resin and elution with 5% (w/v) acetic acid and 0 45% (w/v) NaCl The activity associated with the final peak showing absorption at 254 nm was collected and desalted by passage through Sephadex G-25 Three separate bulk preparations were made and tested for their effects on the accumulation of total leukocytes in sponges implanted subdermally in the rat[7] The results (Table 1) show that the preparations produced a significant reduction, approximately 60%, on the migration of the leukocytes into the sponge exudate There is some variability in activity between different preparations of plasma fraction and our normal practice is to define an active fraction as one which reduces either the development of swelling in the carrageenin-induced paw oedema test[2] or the migration of polymorphonuclear leukocytes into the 5 h sponge exudate[7] in the rat by at least 50% When the plasma fraction was heated at 100 °C for 1 h with 1 5 M NH₄OH solution (inactivated plasma fraction) it failed to inhibit the migration of leukocytes into the 5 h sponge exudate

The second set of experiments used the Boyden chamber technique[11] to investigate the directed migration of rat and human leukocytes in vitro This procedure not only allows quantitative assessment of chemotaxis but also investigation of interference with either the release or the action of complement-derived chemotactic factors Polystyrene, disposable chemotactic chambers (Adaps, Dedham, Massachusetts) and Millipore filters with 3 μm pore size were used, and the methods for the assembly, incubation and counting of the cells which had penetrated the filter were as described by Goetzl and Austen[12] The upper chambers contained the leukocyte suspensions and to the lower chambers were added mixtures of serum and zymosan, which had previously been incubated for 1 h at 37 °C to ensure release of chemotactic factors Plasma fraction preparations, tested on the same day as in the sponge experiments (Table 1) were added, in amounts ranging from 0 1 to 0 005 ml, either to the serum–activator mixture, before or after incubation, or to the cell suspension in the upper chamber Random motility of the leukocytes was also studied in the chemotactic chambers by omitting the incubation of the serum with zymosan The results (Table 2) show that when the plasma fraction was added either to the cell suspension or to the serum–activator mixture after incubation there was no significant change in the chemotaxis of either the rat or human leukocytes When the plasma fraction, but not the inactivated plasma fraction, however, was added to the serum before incubation with the zymosan, a significant reduction in the directed migration of the leukocytes was observed This action of the plasma fraction on the release of the chemotactic factors is dose dependent Thus in the rat leukocyte-serum system the action increased from 10% with 0 01 ml of plasma fraction to 36% with 0 1 ml The human cell–serum system is more sensitive and an inhibition in excess of 80% occurred after the addition of 0 02 ml of the plasma fraction

The failure of the plasma fraction to significantly alter the directed migration when added to the cell suspensions in the upper chambers suggested that the plasma fraction did not act

Table 1 Effects of plasma fraction preparations on leukocyte migration into the 5 h sponge exudate in the rat

Group	Number of cells*
Control	29 1±5 9
Plasma fraction	13 0±1 7†
Inactivated plasma fraction	29 5±3 4

*Results given as mean ±s d and expressed as number of cells ×10⁴ per ml of sponge exudate[7] One sponge implanted in each rat which received an intravenous injection of 1 ml of either saline, plasma fraction or inactivated plasma fraction, five animals per group, the experiments were carried out on three separate occasions
†Significant difference (P<0 001) from results of control and inactivated plasma fraction groups

Table 2 Effects of plasma fraction preparations on the release of chemotactic factors from rat and human serum in vitro

Addition to serum (lower chamber)	Addition to cells (upper chamber)	Number of cells	
		Rat	Man
(a) Directed migration experiments in chemotactic chambers*			
Saline	None	2 0±1 2 (12)	1 9±0 8 (9)
Before incubation with zymosan			
Saline	None	14 5±3 4 (12)	23 5±0 9 (15)
Saline	PF (0 1 ml)	12 8±4 1 (9)	26 8±0 3 (6)
PF (0 1 ml)	None	9 3±2 5 (12)†	ND
PF (0 02 ml)	None	11 3±0 7 (9)‡	4 6±1 9 (15)†
PF (0 01 ml)	None	13 3±1 2 (9)	9 6±6 9 (9)‡
PF (0 005 ml)	None	ND	15 2±8 6 (9)‡
IPF (0 1 ml)	None	14 7±0 8 (9)	ND
After incubation with zymosan			
PF (0 1 ml)	None	13 1±2 9 (9)	24 4±1 0 (6)
(b) Random migration experiments in chemotactic chambers§			
Saline	Saline	10 0±0 7 (9)	5 1±0 6 (9)
PF (0 1 ml)	PF (0 1 ml)	9 8±0 6 (9)	5 2±0 1 (9)

*PF, plasma fraction, IPF, inactivated plasma fraction (see Table 1) Leukocyte suspension (1 0 ml) containing 3 to 5×10⁶ cells per ml of either rat or human leukocytes in upper chamber, corresponding serum (0 9 ml), zymosan (50 μg) and either saline, PF, IPF or mixtures of these to give a final volume of 1 0 ml Results given as mean ±s d and expressed as number of cells per high power field, ten such fields were counted in each experiment and number of separate experiments given in brackets
†Significant difference (P<0 01) from results in which no plasma fraction was added to the serum–zymosan mixture before incubation, in which the plasma fraction was added to the serum–zymosan mixture after incubation, in which the plasma fraction was added to the cell suspension in the upper chamber and in which inactivated plasma fraction was used
‡Significant difference (P<0 01) from results in which no plasma fraction was added to the serum–zymosan mixture
§Reaction mixtures as for directed migration experiments except that incubation with zymosan omitted and either saline or plasma fraction added to both chambers
ND, not determined

on the leukocyte cell surface to prevent chemotactic attraction Further evidence that the plasma fraction did not affect leukocyte behaviour and function is provided by the results of the random migration experiments in the chemotactic chambers (Table 2) and from other work in which suspensions of rat and human leukocytes exposed to the plasma fraction showed no changes in the uptake of trypan blue[13], the phagocytosis of either ink or zymosan granules[14], random motility in capillary tubes[15], adherence to glass and rosette formation[16]

We conclude that the plasma fraction exerts a selective action on the release of chemotactic factors, for example, C3a and C5a (ref 17), after activation of the complement cascade by the alternate pathway in vitro This effect may be of some significance in vivo since the effective amounts of the plasma fraction used in the present work are comparable In the sponge experiments (Table 1) 1 ml of the fraction was injected intravenously and may be assumed to have been diluted in the circulation by a factor of 10 In the chemotactic chamber experiments (Table 2) amounts of the fraction were diluted from 1–10 to 1–200 with either rat or human serum in the lower chamber When complement proteins and leukocytes have entered inflammatory exudates a vicious circle is set up because leukocyte lysosomes not only contain a C5-cleaving enzyme[17] but a substance causing a non-immune activation of the complement system[18] More chemotactic fragments may be generated by the interaction of the leukocytes and extravascular complement thus amplifying the inflammatory response through positive feedback mechanisms[18] The persistence of an inflamatory reaction may be largely dependent on the secondary events mediated by leukocytes which have accumulated at the site of the inflammatory insult The anti-inflammatory activity of the plasma fraction in susceptible animal models, that is, those associated with neutrophil emigration such as carrageenin-induced paw oedema, Arthus

reactions and implanted sponges, is explicable on the basis of a selective interference with the release of complement-derived chemotactic fragments This rather specific site of action may be of wider relevance since it has been proposed[19] that a logical approach to anti-inflammatory therapy would be a blocking of any one of the many steps in the chemotactic response of the neutrophil

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Electrophoretic variation in allelozymes related to function or structure?

ATTEMPTS to explain variation at enzyme loci in terms of the function of the protein molecule[1–5] have shown that enzymes characterised by a single physiological substrate are less variable than enzymes which have been shown *in vitro* to act on a number of substrates[5] and that enzymes regulating the flux through a pathway are more variable than enzymes with no regulatory function[3,4] The explanation for this is that different enzymatic forms are favoured in different environments What distinguishes this hypotheses from others concerning the maintenance of protein variation is that it is to a large extent independent of mutation rate, population size, gene flow among populations and whether or not the population is at equilibrium For these reasons it can easily be tested Johnson[4] and Gillespie and Langley[5] analysed separately data from *Drosophila*, small mammals and man Although the collection of data on small mammals and man is more or less complete, both papers, and that by Gillespie and Langley[5] in particular, ignored a number of *Drosophila* studies I have collected electrophoretic data referring to 61 species and semispecies of *Drosophila* (Fig 1) and carried out an analysis identical to that of Johnson[4], supporting the observation that enzymes with multiple substrates are more variable than enzymes with single substrates Also, enzymes with variable substrates are more polymorphic than regulatory enzymes, which in turn are more polymorphic than non-regulatory enzymes

This analysis (see Fig 1) does not take into account the distribution of variation within the classes mentioned above

Figure 2 gives this distribution for the 260 hydrolases and for the 133 esterases in particular, 23 % of the hydrolases and 21 % of the esterases are monomorphic In most of the studies cited in Fig 1, several populations were examined and all were found to be identically monomorphic at a number of loci with esterase, phosphatase and peptidase activity[6,11,16–18] When closely-related species were studied it was found that this conservatism transcends the species limits[6,22] It seems that about one-fifth of the loci specifying enzymes with hydrolytic activity constitute some of the most conservative loci studied in *Drosophila*, yet they are in the class of multiple or variable substrates

Both α glycerophosphate dehydrogenase (αGPDH) and malic dehydrogenase (MDH) are classified as non-regulatory and by implication their evolution has been guided by the same type of selection forces[4] But αGPDH is consistently poorer in variation than MDH (see Fig 1) Table 1 shows how non-regulatory enzymes change within three groups of closely related species Within the *willistoni* group only two species share the same major allele at MDH, all the others being almost fixed for different alleles No differentiation took place, however, at αGPDH This marked difference repeats itself in the other two groups and it is obvious that when looked at over periods of millions of years, MDH and αGPDH have followed different evolutionary patterns

The classification of enzyme loci according to their function evidently cannot account for the pattern of their variation, although a pattern may arise when heterogeneous data are pooled One reason may be that the assignment of a locus to a

Table 1 Number of major alleles observed within a group of closely related species of *Drosophila*

Enzyme	Group		
	willistoni (6 species)	*obscura* (11 species)	*repleta* (12 species)
MDH	5	9	8
αGPDH	1	3	1
Aldolase	1	–	3
Fumarase	2	–	2
Isocitric dehydrogenase	1	6	–
Triose phosphate isomerase	3	4*	–

* Studied in ten species
Data for the *willistoni* group from Ayala and Tracey[2,3] and Ayala *et al*[24], for the *obscura* group from Lakovaara and Saura[22], and for the *repleta* group this study

multiple substrate group is based on the *in vitro* behaviour of the enzyme, which may indicate nothing about its physiological role This remains unknown for the majority of these non-specific enzymes Contrary to their ability to utilise a wide range of substrates *in vitro*, their function *in vivo* may be quite specific *EstA* of the olive fruit fly *Dacus oleae* behaves *in vitro* like an acetylcholinesterase[25], flies heterozygous for a 'null' allele are less resistant to organophosphate insecticides, and the enzyme is present in the head and the thorax, but not in the abdomen[26] The evidence, therefore, is convincing that *EstA* is associated with the nervous rather than the digestive system of the insect If so, it is difficult to see to what kind of environmental variability this enzyme has been exposed Yet, *EstA* is one of the most polymorphic loci described so far more than fifteen alleles have been detected and in a population of *D oleae* as much as 76 % of the individuals are heterozygous[27]

Far more variation has been observed in some esterases and far less at αGPDH than in loci of other enzymatic functions This observation can be explained by postulating that the most important single factor determining the extent of electrophoretic variation is the primary structure of the protein molecule The primary structure determines the extent directly and not through the functional properties of the enzyme and the environmental diversity to which it is exposed King[28] argued that, provided the size of the population is maintained above a
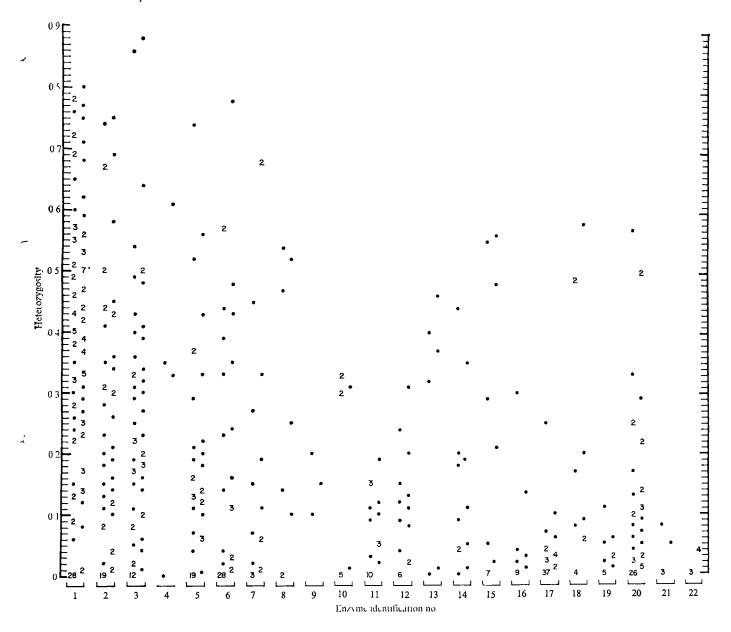
Fig 1 Estimates (635) of hererozygosity at a number of *Drosophila* loci The following species were included in the analysis *affinis²*, *aldrichi⁶*, *ananassae⁷*, *arizonensis⁶*, *athabasca²*, *bipectinata⁸*, *busckii⁹*, *equinoxialis¹⁰*, *maleikothana⁸*, *melanogaster²*, *mojavensis⁶*, *mulleri⁶*, *obscura¹¹*, *pachea¹²*, *parabipectinata⁸*, *paulistorum¹³* semispecies *A*, *AB*, *I* and *T*, *pavani¹⁴*, *persimilis¹⁵*, *pseudoobscura¹⁶*, *robusta¹⁷*, *simulans²*, *subobscura¹⁸*, *tropicalis¹⁹*, *willistoni²⁰*, and 34 species of the genus *Drosophila* in Rockwood *et al* ²¹ Identification numbers stand for 1, esterase, 2, phosphatase, 3, peptidase, 4, amylase, 5, octanol dehydrogehase, 6, alcohol dehydrogenase, 7, aldehyde oxidase, 8, adenylate kinase, 9, glyceraldehyde-3-phosphate dehydrogenase, 10, glucose-6-phosphate dehydrogenase, 11, hexokinase, 12, malic enzyme, 13, phosphoglucose isomerase, 14, phosphoglucomutase, 15, xanthine dehydrogenase, 16, aldolase, 17, αGPDH, 18, isocitric dehydrogenase, 19, fumerase, 20, malate dehydrogenase, 21, 6-phosphogluconate dehydrogenase, 22, triose phosphate isomerase Numbers in the body of the Figure indicate the number of times the corresponding value of heterozygosity has been observed at a given enzyme locus, dots indicate this value has been observed once The amount of variation at a given locus is taken to be independent of the amount of another locus of the same or other species The multiple substrate group of Gillespie and Langley⁵ includes loci from No 1 to No 7 The single substrate group includes the rest of the loci The Wilcoxon two-sample rank test performed on the heterozygosities at these loci gives u_{1-P} = −7 82 According to Johnson⁴, loci from No 1 to No 5 fall in the group variable substrate, loci from No 6 to No 15 in regulatory, and the rest in non-regulatory enzymes Regulatory against variable substrate gives u_{1-P} = −5 24, and non-regulatory against regulatory, u_{1-P} = −3 93 αGPDH against MDH gives u_{1-P} = −3 44 In all tests (corrected for ties) $P<0.001$ Loci from No 1 to No 4 constitute the class of hydrolases

certain limit, the electrophoretic variation at a protein locus is determined by the number of lysine, arginine, glutamic, and aspartic acid residues and, to a lesser extent, by the probability that a conformational change will not affect the protein function Given that this property remains basically unchanged and the composition in the four amino acids mentioned above varies little from species to species, we expected (and found¹⁶,¹⁹ ²⁴) the same amount of polymorphism at homologous loci over populations of the same and related species The differences we observe may be attributed to sampling error or

to genetic drift Indeed, the heterozygosity distribution of esterases (Fig 2), excluding the zero class, is close to normal, as would be expected if these 105 esterases were drawn from the same Universe (When the expected numbers are calculated on a 10% heterozygosity interval, a test for normality gives $\chi^2 = 4.79$, d f = 7, $0.60<P<0.70$)

The hypothesis predicts that structurally related enzymes will show comparable amounts of variation Hydrolases represent such a group Excluding the zero class, the distribution of heterozygosities at the remaining 200 hydrolases does not
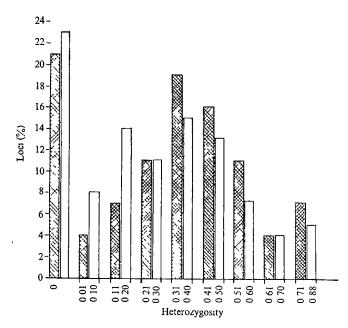
Fig. 2 Distribution of heterozygosities Open bars, hydrolases, shaded bars, esterases

deviate significantly from normal ($\chi^2 = 11 38$, d f = 7, 0 10 < P < 0 20) Hydrolases with zero heterozygosity may represent a separate class It is possible that these conservative esterases, phosphates and peptides are closer to each other in terms of evolution than they are to esterases, phosphatases and pepidases which constitute the polymorphic fraction of hydrolases Oppenoorth and van Asperen[29][30] observed that a single mutation altered an aliesterase of the house fly into an enzyme with phosphatase activity This observation demonstrates the possibility that enzymes of different function may be structurally much closer to each other than they are to enzymes with the same function, especially when this function is determined by a nonspecific substrate

This explanation of electrophoretic polymorphism is compatible with the hypothesis of neutrality[31], since it attaches no significance to the environmental variability Both function and polymorphism are determined by the primary structure of the molecule, and thus seem to be correlated, although this correlation is not a causal one The term allozymes, coined by Prakash *et al*[16] to denote the allelic forms of an enzyme locus, is not entirely proper The greek αλλος for 'other' or 'foreign' does not imply relation The proper word would be allelozymes

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Biosynthesis of alkyl sulphides by an ant

ALKYL sulphides are relatively common in microorganisms and higher plants[1,2], but rare in animals In the arthropods they seem to be restricted to the African stink ant *Paltothyreus tarsatus*[3,4], where they are produced in the mandibular glands and function as releasers of digging behaviour The two sulphides produced by the ant are dimethyl disulphide and dimethyl trisulphide[5] An investigation of the biosynthesis of these compounds seemed particularly interesting for several reasons their *de novo* production in animals is unknown, they seem to be unique to *Paltothyreus* and biosynthetic studies of very few arthropod pheromones have been undertaken

Although microorganisms and plants such as onions, chives and garlic are known to produce both dimethyl disulphide and dimethyl trisulphide[1,6], the biosynthesis of these compounds has not been extensively investigated In plants dimethyl disulphide is known to be produced from methionine as a byproduct of ethylene production[7], and in microorganisms[8] and fungi[9] by oxidative deamination and demethiolation of methionine Since these biosynthetic studies suggested that it was the starting material for sulphide biosynthesis, methionine was chosen as the likely material from which *Paltothyreus* might synthesise its sulphides Insects were previously known only to utilise methionine for trans-sulphuration to cysteine by way of cystathionine pathway[10]

Paltothyreus workers fed on a diet of termites (*Macrotermes natalensis*) were injected with solutions containing L-[35]S-methionine, DL-(1-[14]C)-methionine and L-(methyl-[14]C)-methionine The ants were maintained in the laboratory for 4 d after

Table 1 Incorporation of the label from methionine into *Paltothyreus* sulphides

Labelled methionine	Activity administered (d p m)	Activity in disulphide (d p m)	Activity in trisulphide (d p m)
L-([35]S)-	$2 5 \times 10^6$	1×10^4	$5 7 \times 10^4$
DL-(1-[14]C)-	$2 2 \times 10^6$	0	0
L-(methyl-[14]C)-	$2 1 \times 10^6$	$6 1 \times 10^2$	$2 4 \times 10^3$

injection They were then decapitated, their mandibular glands removed and extracted with methylene chloride This extract, containing the sulphides, was fractionated using gas chromatography and the di- and tri-sulphides individually collected by preparative gas chromatography The fractionated di- and tri-sulphides were then each placed in vials containing 15 ml of Packard Instagel scintillant and their radioactivity determined in a Beckman scintillation counter Table 1 shows that *Paltothyreus* can utilise the sulphur in methionine for the production of the sulphides In addition, the methyl groups in the sulphides seem to arise from the methyl group of the methionine since labelled [14]C is incorporated into the sulphides only when it is in the terminal methyl group

This work provides the first experimental evidence for the *de novo* biosynthesis of sulphides in an arthropod The dimethyl disulphide is probably produced from methionine in much the

$$HOOC.CH(NH_2).CH_2.CH_2.S.CH_3$$

$$\downarrow$$

$$HOOC.CO.CH_2.CH_2.S.CH_3 \quad + \quad NH_3$$

$$\downarrow$$

$$HOOC.CO.CH_2.CH_3 \quad + \quad HS.CH_3$$

$$\downarrow \qquad\qquad\qquad \downarrow$$

$$CO_2 \quad + \quad H_2O \qquad \tfrac{1}{2}(CH_3.S.S.CH_3)$$
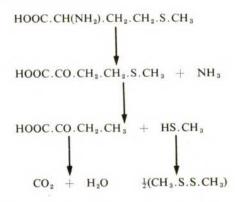
Fig. 1 Possible route for the biosynthesis of dimethyl disulphide from methionine.

same way (Fig. 1) as has been found in microorganisms[8,9]. In the case of the trisulphide the situation is more complex, since even if the two methyl groups and their attached sulphur atoms come from methionine, the origin of the third sulphur atom remains unknown. To determine whether the methanethiol group from methionine is incorporated directly into both the disulphide and the trisulphide, further experiments using methionine doubly labelled with [3]H and [35]S are being undertaken.

Whatever the uncertainties about the precise biosynthetic pathways utilised for sulphide production in *Paltothyreus*, it is clear that methionine can be utilised as the starting material for the biosynthesis of both exocrine products.

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Subunit structure of chromatin is the same in plants and animals

ALTHOUGH plants and animals diverged from each other nearly 10^9 yr ago, the histone composition of their chromosomes is remarkably similar[1]; in particular, the arginine-rich histones III and IV of peas and cows differ by only four and two amino acids, respectively[2,3]. Such extreme evolutionary conservation of a protein sequence argues that histones are crucial to chromosome structure and/or function, and further suggests considerable similarity, at the molecular level, between the chromosomes of plants and animals. We present evidence here for such a similarity.

In animals, chromatin seems to be organised into subunit structures, involving several hundred base pairs of DNA and small numbers of each class of histones[4-5]. Important evidence for a model of this type, in which subunits are arranged as beads on a string, is that, after nuclease digestion of the chromatin, most chromosomal DNA can be isolated as pro-

tected fragments which are integral multiples of a monomeric fragment, 100–200 base pairs long[5-8]. We have done similar nuclease digestions of plant chromatin and have obtained similar results.

Chromatin was isolated from pea seedlings (*Pisum sativum*) by a simplification of the method of Bonner *et al.*[9], using a low ionic strength buffer previously shown to inhibit histone migration[7]. After incubation of the crude chromatin with various concentrations of micrococcal nuclease, the remaining DNA was purified and subjected to agarose gel electrophoresis as described in the legend to Fig. 1. As Fig. 1*d* shows, when pea seedling chromatin was treated exhaustively with nuclease, a discrete DNA fragment remained intact (even when free radioactive DNA or RNA added to the digestion mixture was
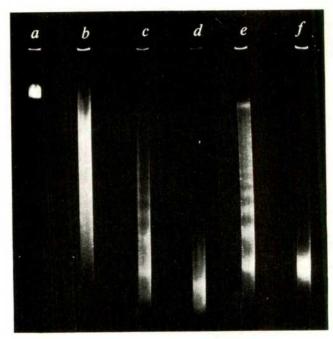
Fig. 1 Agarose gel electrophoresis of: λ DNA (*a*); DNA purified from crude pea chromatin after incubation with 0, 10, and 100 μg ml^{-1} of micrococcal nuclease, respectively, as described below (*b*, *c* and *d*); DNA isolated from a partial digest of mouse myeloma chromatin (*e*); peak fraction from a sucrose gradient of DNA purified from extensively digested mouse myeloma chromatin (and run as a standard in all sets of gels) (*f*). Gels were prepared from 1.4% agarose and run in a solvent of 0.04 M Tris, 10^{-3} M EDTA, pH 7.8, plus 0.1% ethidium bromide[13]. The dye, when intercalated into the DNA, fluoresces when the gel is examined under ultraviolet light. Pea seeds were germinated in the dark, and the shoots collected, washed, frozen, ground with 2 volumes of cold buffer (5×10^{-3} M sodium phosphate, 2.5×10^{-5} M CaCl$_2$, pH 7.0)[7] and blended for 60 s. The extract was filtered through eight layers of cheesecloth and centrifuged at 4000*g* for 30 min at 0–4 °C. The crude chromatin pellet was removed from the underlying starch pellet and resuspended in 1/50 to 1/100 of the original volume of buffer. Micrococcal nuclease (Worthington) was added to various concentrations and incubated at 37 °C. After 20 min, ribonuclease was added to 0.2 mg ml^{-1}, and at 30 min EDTA, sodium dodecyl sulphate and nuclease-free pronase were added to final concentrations of 10^{-3} M, 0.1% and 0.5 mg ml^{-1} respectively. The mixtures were incubated a further 30 min at 37 °C and then extracted two or three times with phenol. Phenol was removed either by ether extraction followed by bubbling with nitrogen, or by passage twice through a short column of P-2 (BioRad). The bands in the gels disappeared on incubation with DNase I, DNase II and micrococcal nuclease, but not with RNase, pronase, α-amylase or cellulase. Thermal denaturation indicated that the purified DNA was native, with a base composition close to that of pea seedling DNA. The same digestion patterns were seen with several minor variations of the above protocol; for example, using a lower ionic strength buffer (10^{-3} M Tris, 2.5×10^{-5} M CaCl$_2$, pH 8.4), extracting with a Dounce homogeniser rather than blending, or adding 1–2 mg ml^{-1} of α-amylase at the same time as the ribonuclease. Similar digestion patterns have been obtained with chromatin isolated from the shoots of black-eyed peas (*Vigna sinensis*, unpublished)

rendered greater than 95% acid soluble). Comparison of the amount of DNA in Fig. 1*d* with that of the no-enzyme control (Fig. 1*b*) indicates that more than half of the isolated DNA migrated as this protected fragment. Moreover, the size of this fragment, as estimated from the distance migrated in the gel, was close to that of a DNA fragment obtained from a digest of nuclei isolated from mouse myeloma cells (Fig. 1*f*; using a method similar to ref. 6; J.D.M. and Kimmel, C. B., unpublished). With less extensive nuclease treatment of the pea chromatin, a series of DNA bands (at least seven) was obtained (Fig. 1*c*), corresponding closely to DNA bands seen in a partial digest of either mouse nuclei (Fig. 1*e*) or calf thymus chromatin (kindly provided for comparison by B. R. Shaw and K. E. Van Holde). When the logarithm of the band number (counting back from the fastest running band) is plotted against the distance migrated in the gel (Fig. 2), the plot is linear, indicating that the length of the DNA in any particular band is an integral multiple of the length of the DNA in the monomer band. Furthermore, the data from pea, calf (not shown) and mouse digests fall on close, parallel lines, indicating similar fragment sizes. From the differences of the intercepts of least squares lines fitted to such
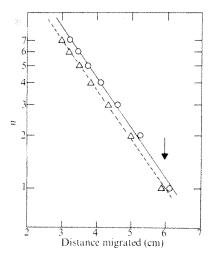
Fig. 2 Plot of the logarithm of the band number (n) against distance migrated in the gel: ○, pea chromatin digest; △, mouse myeloma chromatin digest. Distances were averaged from four to nine densitometer traces of film negatives of the gels (varying gel position, film exposure times and so on), and each point is the average of three parallel gels. The arrow indicates the migration position of the sucrose gradient fraction from a mouse digest used in Fig. 1*f*, above, which has been shown to be 170 ± 30 base pairs, as measured by sedimentation velocity. The lines represent the least-squares best fit of the data to the equation: $\log(n) = a \cdot (\text{distance migrated}) + b - \log(\text{size of monomer fragment})$.

data, the ratio of the length of the monomer DNA fragment in pea seedling chromatin to that in mouse chromatin can be estimated as 0.95 ± 0.2 (weighted average from 14 gels). We estimate the absolute size of the monomer DNA fragment as 170 ± 30 base pairs, based on the gel migration of a purified mouse myeloma DNA fraction (Fig. 1*f*) which had a sedimentation coefficient ($S^0_{20,w}$) of 5.5 ± 0.3 as determined by analytical band sedimentation[10,11]. This size estimate lies between those previously made on DNA fragments isolated from rat liver[6] and calf thymus chromatin[5,7]. Although we have not seen a discrete submonomer band (as has been described by Noll[6] in a digest of rat liver chromatin), as Fig. 1*c* shows, some DNA migrated ahead of the monomer band. Furthermore, after prolonged nuclease treatment (for example, Fig. 1*d*) most of the DNA migrated to a position in the gel corresponding to a size 80% of the monomer band, or about 140 base pairs (the $S^0_{20,w}$ of the DNA sample used in Fig. 1*d* was 5.0 ± 0.3, corresponding to a DNA length of 125 ± 20 base pairs).

As with similar studies on mammalian systems, the simplest model to explain our results is that a specific histone–DNA

complex protects discrete lengths of DNA from nuclease digestion; thus the fundamental architecture of chromosomes may well be the same in both plants and animals. Since, however, several of the histone classes do show differences between different organisms[1], and yet, at least at the level of this study, their combined effect on DNA is quite similar, comparative studies (potentially leading to inter-species reconstitution experiments) seem an attractive approach to the investigation of the detailed structure of the chromatin particles, and the forces involved in its maintenance. As a recent example of such a comparative study, nuclease digestion of yeast chromatin (which lacks certain classes of histones) was found to give a protected DNA fragment which was both smaller and more homogeneous than that obtained from calf thymus chromatin[12]. A number of questions are suggested. When did this subunit structure arise in evolution? What purpose does this chromatin particle serve that requires such a specific length? If it represents simply a structural means to package large amounts of DNA, why is it so unusual that it has been stringently conserved throughout the course of evolution?

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Age-dependent excision repair of damaged thymine from γ-irradiated DNA by isolated nuclei from human fibroblasts

THE lifespan of diploid human fibroblasts in culture is limited[1], although there are substantial variations in lifespan with different culturing conditions and growth media[2-4]. Before death, the cells pass through a period of senescence, characterised by numerous structural changes[5]. According to the error catastrophe hypothesis, a breakdown of the fidelity of the expression of genetic information resulting in the production of faulty proteins[6-11], and the accumulation of mutations in the genome[12], may represent important mechanisms of cellular ageing. It would be predicted that the deterioration of the quality of the genome would be accelerated by deficient or error-prone DNA repair. Several steps in the excision repair of damaged DNA residues have been demonstrated in mammalian cells exposed to exogenous physical or chemical agents. In the case of ionising radiation, the removal from the DNA of thymine damaged by γ rays was observed in Chinese hamster ovary[13] and human WI–38 cells[14], and the filling of the resulting gaps, indicated by repair replication or unscheduled synthesis, has been demonstrated in numerous cell lines[15]. Recently published experiments[16] have demonstrated that the capacity of skin fibroblasts to perform ultraviolet-induced repair synthesis
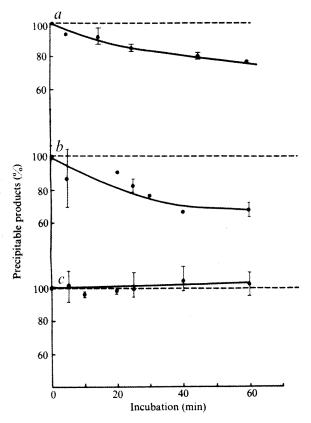
Fig. 1 Excision of thymine radiation products of the t' type from γ-irradiated PM2 DNA by isolated nuclei from (a) young (passage 23), (b) medium-aged (passages 37 and 39) and (c) old cells (passages 43, 47 and 48). Diploid human lung fibroblasts WI-38 were grown in tissue culture according to the method of Hayflick and Moorhead[1]. Isolated nuclei were prepared by a modification of the procedure of Berkowitz *et al.* (ref. 21 and J. F. Remsen and P.A.C., unpublished). Cells were collected by trypsinisation into sterile screw-cap bottles (50 ml), washed twice with ice-cold Hanks' balanced salt solution, and centrifuged at 1,200 r.p.m. for 10 min. Homogenisation was then effected in

hypertonic sucrose buffer (0.32 mM sucrose, 0.3 % Triton X-100, 1 mM potassium phosphate buffer (*p*H 7.1), 1 mM Mg²⁺, 1 mM dithiothreitol) with a Dounce homogeniser, after which the suspensions were centrifuged at 1,200 r.p.m. for 10 min. The homogenisation procedure was repeated and, following centrifugation, the nuclei were washed twice in isotonic sucrose buffer (0.25 M sucrose, 1 mM potassium phosphate buffer (*p*H 7.1), 1 mM dithiothreitol, 3 mM Ca²⁺) and the nuclear suspensions centrifuged. In some experiments, nuclei were disrupted by sonication (two treatments for 10 s at the lowest setting) and the completeness of sonication checked using phase contrast microscopy. Pseudomonas phage PM2 DNA labelled with ³H-methyl-thymidine was prepared according to Franklin *et al.*[22], and purified on hydroxyapatite before each experiment. This was used as DNA substrate for the experiments using γ-irradiation because it could be prepared essentially free of strand breaks. Irradiation was carried out under non-protective conditions in aerated phosphate buffer with a ¹³⁷Cs-source; doses used (usually 15 to 25 krad) resulted in the formation of 0.3–0.6% t', as determined in the acid-precipitable material by the alkaline–acid degradation assay of Hariharan and Cerutti[23]. The DNA was passed through a small Sephadex G75 column following irradiation to remove a small amount of low molecular weight material. Although t' represents a major class of radiation products, γ-irradiated DNA contains, in addition, a variety of products derived from the other nucleic acid bases, apyrimidinic[24] and, possibly apurinic sites, as well as radiation-induced strand breaks. A second substrate containing primarily 5,6-dihydroxy-dihydrothymine and a small number of apyrimidinic sites (B. E. Dunlap and P. A. C., unpublished) was therefore prepared by the oxidation of polyd(A-T)–³H-methylthymine with OsO₄. The conditions of Beer *et al.*[25] were used and the oxidation was allowed to continue until the polymer contained 0.3–0.7% t', or 2–4.5% ring-damaged thymine[23]. The samples for the excision experiments contained (in a total volume of 150 μl) 4 × 10⁶ nuclei or sonicated nuclear equivalents, labelled (0.5–1.5 × 10⁵ c.p.m.) irradiated PM2 DNA or OsO₄-oxidised polyd(A-T) corresponding to 1–3 × 10⁻⁶ μmol t', 0.01 M phosphate buffer (*p*H 7.6), 0.09 M NaCl, 1 mM dithiothreitol, 15 U pyruvate kinase, 15 mg ml⁻¹ BSA (Fraction V), 2 mM PEP, 0.4 mM ATP, and 0.2 mM of each of the four deoxynucleoside triphosphates. Incubation with substrates was carried out for up to 60 min at 37 °C and the reactions terminated by the addition of 1.9 ml 7% trichloroacetic acid. Thymine radiation products (t') were determined using the above method of Hariharan and Cerutti[23]. The ratio of t' in the acid-precipitable material over the total acid precipitable input counts (t'/Tppt) was computed for each time point and expressed as the percentage of the corresponding value for a non-incubated control sample.

increases as a function of the lifespan of the donor species. We report that isolated nuclei or nuclear sonicates from senescent diploid human lung fibroblasts WI–38 have lost their ability to excise from DNA thymine damaged by γ rays.

We have studied the capacity of isolated nuclei and nuclear sonicates from young, medium-aged and old WI–38 cells to excise damaged thymine residues of the 5,6-dihydroxy-dihydrothymine type (t') from exogenous γ-irradiated or osmium tetroxide (OsO₄) oxidised DNA substrates. Our experimental design allows the use of non-irradiated and unlabelled nuclei. Comparison of results obtained with these two substrates allows an assessment of the effects of damage other than t', in particular, radiation-induced strand breaks, on the repair process (see legend to Fig. 1). The loss of t' from the acid-precipitable exogenous DNA substrate and the amount of undamaged thymine rendered acid-soluble were determined as a function of incubation time.

WI–38 cells of passages 14 and 29 (L. Hayflick, Stanford) were used as starting cultures for the production of cells at the three age groups investigated. Under our culturing conditions, cell death usually occurs at passages 49–52. The age in culture of the WI–38 cells from which the nuclei were prepared is reflected in the passage number (that is, the number of 1:2 subculturings) and was further characterised by the percentage of nuclei which were labelled in a 48 h pulse with ³H-methyl-thymidine according to the method of Cristofalo and Sharf[17]. These authors found a decrease with passage number in the percentage of nuclei actively synthesising DNA within a 48 h period, starting approximately at passage 30, and suggested that such data could be used for the character-

isation of the functional state of WI–38 cells. Their results and ours are in agreement. In the following, we refer to cells at passages 15–25 as 'young', to cells at passages 26–42 as 'medium aged', and to cells at passages above 42 as 'old'.

Figure 1*a* shows that young nuclei of passage 23 and medium-aged nuclei of passages 37 and 39 removed approximately one-quarter to one-third of t' from the γ-irradiated acid-precipitable PM2 DNA within 60 min of incubation at 37 °C; however, no excision of t' was accomplished by old nuclei of passages 43, 47 and 48. Only 2–6% of total thymine label was rendered acid-soluble during 60 min incubation. Excision of t' by nuclei from young and medium-aged cells remained incomplete. The percentage excision within 60 min of incubation is comparable to that accomplished by human skin fibroblasts at similar concentrations of t' (J. F. Remsen, P. V. Hariharan and P. A. C., unpublished). Analogous data (not shown) were obtained for the excision of t' from γ-irradiated PM2 DNA after incubation with nuclear sonicates. The capacity of WI–38 nuclei to excise t' from OsO₄-oxidised polyd(A-T) also depended on cell age: WI–38 nuclei obtained from young cells at passages 21 and 24 selectively excised 28% of t' within 60 min of incubation at 37° C; nuclei from medium-aged cells at passage 34 excised 22% of t'; no excision was observed with old nuclei of passage 47. The excision of t' from OsO₄-oxidised polyd(A-T) by young and medium-aged nuclei occurs with very high selectivity, the acid solubilisation of thymine label within 60 min of incubation being only 0.6–1%.

The following conclusions can be drawn from our results: Young and medium-aged nuclei up to a passage number of

approximately 42 (corresponding to a percentage labelled nuclei value of 60-70%)[17] possess the capacity for the selective excision of γ-ray damaged or OsO_4-oxidised thymine (t') from exogenous DNA. The fact that excision occurs from OsO_4-oxidised polyd(A-T) indicates that neither damage to bases other than thymine nor, in particular, radiation-induced strand breakage is required for removal of t' from DNA. The extent of polymer degradation which accompanies excision is small, particularly for OsO_4-oxidised polyd(A-T). In this case, only 1.6 undamaged nucleotides are released per ring-damaged thymine within 60 min of incubation. Old nuclei do not accomplish excision of t' either from γ-irradiated PM2 DNA or OsO_4-oxidised.polyd(A-T). The loss of the excision capacity in old nuclei and nuclear sonicates occurs within a few generations (passages 42 to 45)—at least four to five generations before cell death occurs—and seems to occur more precipitously than the loss of the capacity for DNA synthesis, as measured by the percentage labelled nuclei method of Cristofalo and Sharf[17]. The loss of repair enzymes during the preparation of nuclei from old but not from young or medium-aged cells could also explain our findings.

The observed loss of the excision repair capacity for γ-ray-induced thymine damage by nuclei from senescent WI-38 cells does not allow conclusions concerning the validity of error catastrophe hypotheses of cellular ageing[10-12]. Because the cells were found to survive for several generations after the repair deficiency had first manifested itself, however, the loss of this function does not seem to be a direct consequence of cell death. It is, on the other hand, impossible, on the basis of our data, to decide whether the observed repair deficiency is one of the causes or a consequence of senescence in cultured WI-38 cells. Painter and collaborators[18,19], found a decline in the ability of WI-38 cells to undergo ultraviolet or γ-ray-induced repair replication only in the last one or two passages before cell death occurred. Hart and Setlow[20] observed a concomitant decrease in the capacity for ultraviolet-induced unscheduled synthesis and scheduled DNA synthesis in senescent WI-38 cells. We have, however, investigated an earlier step in γ-ray excision repair than in those studies measuring unscheduled synthesis or repair replication.

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Determination of recognition sites of T4 RNA ligase on the 3'-OH and 5'-P termini of polyribonucleotide chains

RNA LIGASE which was discovered by Silber *et al.*[1] in T4-infected *Escherichia coli* cells, catalyses an ATP-dependent, intramolecular joining reacion of 5'-P and 3'-OH termini of various homopolyribonucleotides[1,2]. The shortest circularising polyadenylate was shown to be $(pA)_8$, the optimal chain length for the reaction being 10–30 (ref. 2). These facts suggest that the substrate for T4 RNA ligase is a pair of suitably juxtaposed, 3'-OH and 5'-P termini, each a few single stranded residues long[2].

An intermolecular joining RNA ligase reaction can be induced by suitably juxtaposing termini of different polyribonucleotide chains. Thus a pair of $tRNA^{Phe}$ half-molecules, fragments 1–36 and 38–74, could be joined by RNA ligase at the termini of the severed anticodon loop[3]. To effect intermolecular joining reactions of non-complexed polyribonucleotide chains, however, other means must be sought to juxtapose their termini, such as increasing the polyribonucleotide concentration (O. C. Uhlenbeck, personal communication). We have therefore investigated the minimal recognition sites on the 3'-OH and 5'-P termini of RNA ligase substrates. We have tested the ability of short oligonucleotides varying in chain length and carrying only one functional terminus to participate in RNA ligase-catalysed reactions. As oligonucleotides carrying only a 3'-OH function, we generally used $(Ap)_nA$ where $n \geqslant 4$, while

Fig. 1 A, Joining of $(Ap)_nA$ with 5' ^{32}P $(pA)_7$. 5' ^{32}P-labelled oligoadenylates, $(Ap)_nAs$ and RNA ligase were prepared as before[2]. The reaction mixtures (0.01 ml) contained: 5' ^{32}P $(pA)_7$ (2,000 c.p.m. pmol^{-1}) 0.65 μM, Tris-HCl buffer pH 7.5, 50 mM; $MgCl_2$ 10 mM, dithiothreitol 1 mM, ATP 0.5 mM, $(Ap)_nA$ as indicated 2 mM, and 0.05 U of RNA ligase. The reaction mixture was incubated at 37 °C for 3 h. An aliquot of 5 μl was then mixed with 5 μl of 50% sucrose, 0.1 M Tris-borate, pH 8.3, 0.01 M EDTA, 0.01% bromophenol blue, applied to a 20% polyacrylamide 7 M urea gel slab and separated by electrophoresis as previously described[2]. *a*, $(pA)_7$; *b*, reaction mixture with no $(Ap)_nA$ added; *c*, ApA added; *d*, $(Ap)_2A$ added; *e*, $(Ap)_3A$ added; *f*, $(Ap)_4A$ added; BB, bromophenol blue R_{BB} values of $(Ap)_nAs$ were obtained by separating a mixture of $(Ap)_nA$ ($n = 4$–17) on the same gel. The bands were detected by staining with 'Stains-all' pure $(Ap)_4A$ served as a reference band. B, Joining of $(Ap)_nA$ with 5' ^{32}P $(pA)_{10}$. The reaction mixtures were essentially as described in (*A*) except that $(pA)_7$ was substituted by 1.5 μM 5' ^{32}P $(pA)_{10}$.

periodate-treated $(pA)_n$ oligonucleotides ($n = 2,3,4$) served for study of the substrate requirements at the 5'-P terminus.

Two kinds of assay were carried out: first, linear joining of either kind of the short monofunctional oligonucleotide to one of the termini of a 5'-[32P] $(pA)_7$, and second, their competition with $(pA)_{10}$ circularisation. The shortest circularising polyadenylate was previously shown to be $(pA)_8$ (ref. 2). A hepta-adenylate will not circularise. In the presence of RNA ligase and excess of short dephosphorylated oligo As, however, $(pA)_7$ participated in a linear joining reaction. When samples of these reaction mixtures were analysed by polyacrylamide-urea gel electrophoresis, it was found that, when reacted, $(pA)_7$ was converted into a longer oligonucleotide migrating like a corresponding $(Ap)_{7+n}A$ marker (Fig. 1A). This oligonucleotide was therefore the linear joining product of $(Ap)_nA$ with $(pA)_7$. ApApA was the shortest oligonucleotide to support such a reaction while ApA had no effect. High concentrations of $(Ap)_nA$ were required to elicit linear joining (Fig. 2) at rates
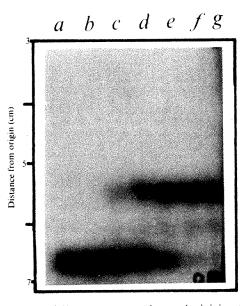
Fig. 2 Effect of $(Ap)_3A$ concentration on its joining to $(pA)_7$. The reaction mixtures (0.04 ml) contained 5' [32P] $(pA)_7$ 0.5 μM, Tris HCl, pH 7.5, 50 mM; MgCl₂ 10 mM, dithiothreitol 1 mM, ATP 0.5 mM, $(Ap)_3A$ as indicated and 0.5 U of RNA ligase. The mixture was incubated at 37 °C; after 2 h 5 μl aliquots were separated by electrophoresis as described for Fig. 1A. *a*, $(pA)_7$; *b*, reaction mixture without $(Ap)_3A$; *c*, 0.075 mM $(Ap)_3A$; *d*, 0.15 mM $(Ap)_3A$; *e*, 0.3 mM $(Ap)_3A$; *f*, 0.6 mM $(Ap)_3A$; *g*, 1.2 mM $(Ap)_3A$.

comparable with those seen in the circularisation of oligoadenylates. In standard assay conditions[2], the apparent K_m of $(Ap)_3A$ (at 5.10^{-7}M $(pA)_7$) was about 8.10^{-4} M, about 1,000 times higher than the K_m of a well-circularising substrate[2] with a pair of 3'-OH and 5'-P termini.

Since short, dephosphorylated oligoadenylates were shown to serve as 3'-OH acceptor termini in an RNA ligase reaction, we expected them also to compete with the 3'-OH of a circularising oligoadenylate, such as $(pA)_{10}$, for the 5'-P terminus. As a result, linear rather than circular joining products would be formed. Figure 1B shows the analysis, by gel electrophoresis, of reaction mixtures containing RNA ligase, 5' [32P] $(pA)_{10}$ and a short dephosphorylated oligoadenylate in about 500-fold excess over $(pA)_{10}$. In the mixture containing enzyme and $(pA)_{10}$ only, complete circularisation took place as indicated by the conversion of linear $(pA)_{10}$ ($R_{BB} = 0.85$) into a faster migrating product ($R_{BB} = 0.90$), as previously described[2]. Addition of ApA had no effect, while $(Ap)_2A$, $(Ap)_3A$ and $(Ap)_4A$ induced the formation, in addition to circular $(pA)_{10}$, of two new, more slowly migrating products. Of these, the slower band comigrated with $(Ap)_{10+n}A$ marker, and probably resulted from linear joining of $(Ap)_nA$ to $(pA)_{10}$ in competition

with the circularisation reaction. The yield of the linear joining product of $(pA)_{10}$ and $(Ap)_nA$ comprised about 10% of the original labelled substrate. The slower new product resulted from the linear addition of $(Ap)_nA$ to 5' [32P] $(pA)_8$p contaminant, present in the $(pA)_{10}$ preparation (see the faster band in slot *a*, Fig. 1B). This contaminating oligoadenylate cannot circularise and is completely converted into a linear joining product in presence of $(Ap)_nAs$.

To study the requirements of RNA ligase at the 5' terminus of its substrate we tested the ability of short, 5' phosphorylated, 3' periodate oxidised, oligoadenylates ($(pA)_n^{Ox}$) in joining to 5' [32P] $(pA)_7$, and in competition with 5' [32P] $(pA)_{10}$ circularisation. Figure 3A and B shows the analysis of products of such reactions by polyacrylamide gel electrophoresis. All the $(pA)_n^{Ox}$ oligoadenylates tested, starting from a dinucleotide, participated in linear joining to $(pA)_7$ and to $(pA)_{10}$, in the latter case at the expense of circularisation. Again, relatively high concentrations of $(pA)_n^{Ox}$ were required to elicit detectable linear joining reactions.

The experiments with dephosphorylated oligoadenylates (Fig. 1A and B) indicated that the RNA ligase recognition site on the 3'-OH terminus consists of no more than a trinucleoside diphosphate (ApApA) stretch. A dinucleoside monophosphate (ApA) is not sufficient. However, a dinucleoside diphosphate cannot be ruled out as a minimal 3'-OH recognition site. Since the experiments with 5'-$(pA)_n^{Ox}$ indicated that $(pA)_2$ and possibly smaller portions of the 5'-P terminus are recognised by RNA ligase, one might expect that $(pA)_2$ would be polymerised by RNA ligase, were it recognised also as a 3'-OH terminus. Our preliminary results (unpublished) confirm this expectation.

The minimal 5'-P recognition site is possibly less than a dinucleoside diphosphate (Fig. 3). It was previously found in the RNA ligase-catalysed loop closure reaction of yeast tRNA[Phe] fragments 1–36 and 38–74 (ref. 3), that no more than one single stranded nucleotide residue (emanating from a double helix) is required for recognition of the 5'-P terminus. Whether the 5'-P recognition site extends beyond the first nucleotide residue to include some portion of the adjacent residue is unknown. The possibility that T4 RNA ligase can join nicked, double stranded
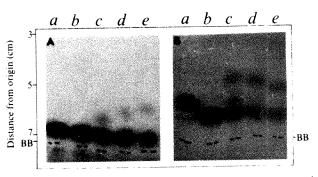
Fig. 3 A, Joining of $(pA)_n^{Ox}$ to 5' [32P] $(pA)_7$. $(pA)_2$, $(pA)_3$ and $(pA)_4$ were prepared by controlled digestion of poly(A) by sheep kidney nuclease[8] followed by fractionation of the digest on a DEAE-cellulose column. To oxidise their 3' ribose these oligonucleotides were treated with sodium periodate as follows: the reaction mixture (0.025 ml) contained 10 mM oligonucleotide and 15 mM sodium periodate and was incubated for 30 min at 0 °C, at the end of which 1 μl of 0.25 M ethylene glycol was added. The solution of the periodate-treated oligonucleotide was used as such in the following experiment. The joining reaction mixture (0.02 ml) contained 4×10^{-7} M $(pA)_7$, Tris-HCl, pH 7.5, 50 mM, MgCl₂ 10 mM, dithiothreitol 1 mM, ATP 0.5 mM, $(pA)_n^{Ox}$ as indicated and 0.1 U RNA ligase. After 2 h of incubation at 37 °C, 3 μl samples were separated by electrophoresis as described in legend to Fig. 1A. 1, $(pA)_7$; 2, reaction mixture without $(pA)_n^{Ox}$; 3, 1.2 mmol of $(pA)_2^{Ox}$ added; 4, 1.2 mmol of $(pA)_3^{Ox}$ added; 5, 0.8 mmol of $(pA)_4^{Ox}$ added. B, Joining of $(pA)_n^{Ox}$ to 5' [32P] $(pA)_{10}$. The reaction mixture was similar to that in *A*, except for containing 1.8×10^{-6} M of $(pA)_{10}$ instead of $(pA)_7$. *a*, $(pA)_{10}$; *b*, reaction mixture without $(pA)_n^{Ox}$; *c*, 0.8 mM of $(pA)_4^{Ox}$ added; *d*, 1.2 mM of $(pA)_3^{Ox}$ added; *e*, 1.2 mM of $(pA)_2^{Ox}$ added.

RNA[1,4] must also be considered; a definitive answer awaits studies with carefully defined substrates.

Our results and those of Uhlenbeck (personal communication) show that T4 RNA ligase can catalyse the joining reaction between unassociated polyribonucleotide chains. In view of the high substrate concentrations required to support such reactions, we consider it unlikely that selective joining reactions of non-complexed RNA molecules are catalysed by the enzyme *in vivo*. The high affinity of RNA ligase for its substrates in the loop closure[3] and circularisation reactions, as well as the competition experiments between intra- and intermolecular joining indicate that a pair of suitably apposed 3'-OH and 5'-P termini of RNA molecules constitutes the *in vivo* substrate of RNA ligase. The requirement that the pair of termini be suitably apposed can be met when (1) they belong to different chains that are partially hybridised, as in severed loops, or (2) when proximity is mediated by a specific factor, or (3) when the termini belong to one chain capable of circularisation. A possibility that cannot be dismissed is that this T4-induced enzyme evolved as a host RNA-modifying function. Other possible participants of such a pathway could be two other early induced T4 enzymes—polynucleotide kinase[5] and the nuclease that specifically cleaves one of the isoaccepting host tRNA[Leu] species[6].

The linear joining reaction catalysed by RNA ligase offers important new synthetic possibilities. It permits joining of tri- and higher oligoribonucleotides in a stepwise manner to a 5'-P terminus of a growing chain. The following scheme is suggested for one step in such a synthesis. First, a dephosphorylated oligonucleotide is joined in an RNA ligase-catalysed reaction to a 5'-P initiator polynucleotide, the 3'-OH terminus of which is blocked. Second, the 5'-OH terminus of the product is phosphorylated by polynucleotide kinase, ready for a subsequent joining step. The synthesis may be programmed in such a way as to generate single stranded chains only; these in turn may be hybridised and subsequently joined in an RNA ligase-catalysed loop closure reaction[3].

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Sequential translation of capsid and membrane protein genes of alphaviruses

INVESTIGATIONS of the multiplication of alphaviruses such as Semliki Forest virus (SFV) or Sindbis virus have suggested that the three glycoproteins[1] (E1, E2 and E3) located in the viral envelope are initially synthesised as a large precursor polypeptide which contains the amino acid sequences of them all[2-7]. This protein, NVP97 (ref. 5), is cleaved to give E1 and another precursor polypeptide, NVP63, which is in turn cleaved to give E2 and E3 (refs 6 and 7). By analogy with the picornavirus system, it has been widely suggested that all the viral structural proteins are derived by cleavage of a common
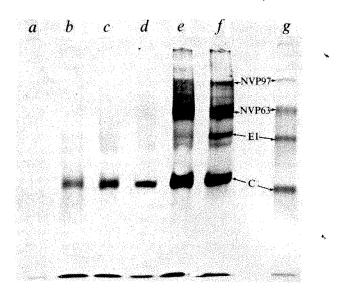
Fig. 1 Monolayer cultures of BHK cells in glass scintillation vials (about 10[6] cells per vial) infected 5.5 h previously with SFV (20 PFU per cell) were exposed to minimal medium (Earle's salts containing 2% dialysed calf serum) to which 225 mM NaCl (ref. 14) had been added. After 40 min the hypertonic medium was replaced with isotonic minimal medium containing [35]S-methionine (200 Ci mmol[-1]; 10 μCi ml[-1]). Actinomycin D (1 μg ml[-1]) was present at all times after infection of the cells. As Saborio *et al.*[14] found, exposure of the cells to the salt-containing medium resulted in cessation of protein synthesis within 15 min. After restoration of isotonicity protein synthesis resumed without delay at a rate nearly equal to that before shutoff. After (*a*) 1 min, (*b*) 2 min, (*c*) 3 min, (*d*) 4 min, (*e*) 5 min or (*f*) 6 min, incorporation was stopped by freezing the cells in dry ice–methanol mixture[11] and adding 5% TCA containing 1% casamino acids. After thawing the cell sheet, washing twice with 5% TCA, and twice with ethanol–ether (1:3 v/v), it was dissolved in 2% SDS, 1% 2-mercaptoethanol, 0.1 M Tris-HCl, pH 9.0. The proteins were alkylated and analysed on 10% polyacrylamide gel slabs, which were dried and autoradiographed[8]. Proteins labelled for 2 min and chased for 12 min in similar cultures of untreated cells (*g*) are shown for comparison. Virus-specific proteins are identified using the nomenclature of Morser *et al.*[5].

precursor and, indeed, proteins of a size sufficient to encompass the viral capsid protein, as well as the envelope proteins, have been found in infected cells treated with amino acid analogues and inhibitors of proteolytic enzymes[10,11], and in cells infected with certain classes of temperature-sensitive mutant[2,3,12,13]. Doubts have been cast on the validity of the analogy, however, by reports[8,9,16-19] that cell-free translation of SFV or Sindbis virus mRNA in several systems resulted in the production of discrete viral proteins. This situation is in marked contrast to that of translation of picornavirus RNA, in which a spectrum of polypeptides of various lengths is formed by premature termination at many points along the messenger; since no post-translational cleavage seems to take place, no polypeptides found in the mature virus or in infected cells are formed[20,21]. The possibility arose, therefore, that the alphavirus capsid protein and envelope proteins are synthesised independently.

Saborio *et al.*[14] have shown that restoring to isotonicity cultures of HeLa cells infected with polio virus, which had been treated with medium containing an elevated concentration of NaCl, induced the synchronous initiation of protein synthesis and allowed the order in which the viral proteins were synthesised to be examined. Here, I report similar experiments with SFV-infected BHK cells, which clearly demonstrate the processes of sequential synthesis and cleavage taking place during the formation of all the alphavirus structural proteins.
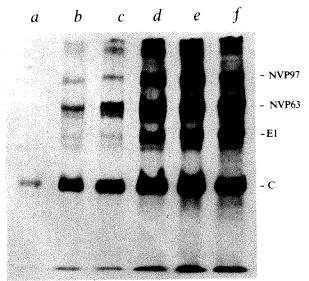
Autoradiographs of SDS–polyacrylamide gel slabs containing the proteins synthesised during a pulse experiment are shown in Fig. 1. By comparison with the proteins synthesised in non-synchronised cells, capsid protein is first apparent 2 min after restoring isotonicity, whereas radioactivity does not become associated with discrete envelope proteins or their

precursors until after 5–6 min, when NVP97, NVP63, and an envelope protein (whose identity is discussed below) all emerge together from the background radioactivity resulting from nascent chains and residual host protein synthesis. If the pulse of radioactivity is followed by a chase period a different picture emerges (Fig. 2). After a 1 min pulse only radioactivity which could be chased into capsid protein had been incorporated. As the length of the pulse increases there is a sequential appearance of envelope-specific material; NVP97 and NVP63 are present after a 2 min pulse and a protein migrating with the envelope proteins of the virus appears after a 4 min pulse. Large proteins of molecular weight greater than 100,000 are also apparent after a 3 min pulse and chase.

The fact that only capsid protein is labelled after a 1 min pulse and chase strongly suggests the existence of a single initiation site at the beginning of the capsid protein cistron for the translation of all the virus structural proteins. If there were two independent initiation sites for the capsid and envelope proteins, products originating from both sites would be expected to be labelled in a pulse-chase experiment, no matter how short the pulse. Other evidence, derived from work on the cell-free translation of 26S RNA, and on the kinetics with which synthesis of different viral proteins is switched off in cells treated with hypertonic medium, is also consistent only with the existence of a single initiation site for the synthesis of all the structural proteins of the virus (J. C. S. C. and S. I. T. Kennedy, unpublished).

From the time of first appearance of discrete envelope protein and precursors in the pulse experiment (Fig. 1), it seems that complete translation of the genes for the structural proteins (total molecular weight about 140,000) takes about 5 min. Assuming a constant rate of ribosome movement, the time required to translate the region corresponding to the capsid protein (molecular weight 36,500) may be estimated to be approximately 1.3 min. The appearance of capsid protein after a 2 min, but not a 1 min, pulse is in agreement with this estimate and indicates that it is cleaved from the growing peptide chain very shortly after translation of envelope-specific material begins. It is clearly not necessary that any precursor protein be completed before this cleavage occurs. The small quantities of high molecular weight proteins which can be seen after a 3 min pulse and chase may represent the precursor which has escaped the capsid protein cleavage mechanism. It is not clear whether these molecules can be processed to give viral

proteins or whether they represent errors which cannot be corrected, but in any event they are certainly not on the main pathway of synthesis of the proteins.

The next proteins to be labelled after the capsid protein in the pulse-chase experiment are the envelope precursors NVP97 and NVP63. The fact that NVP63 is labelled in the absence of radioactivity in the envelope region of the gels indicates that it is NVP63 which is translated immediately after the capsid protein. The absence of labelled envelope protein also means that NVP63 is not cleaved to give observable E2 and E3 during the short chase periods used here. This may mean that NVP63 is an intermediate with a relatively long half life, or that this cleavage is closely connected with the release of virus by budding through the cell membrane, as has been previously suggested[6,15]; if this were the case, significant quantities of E2 and E3 would not accumulate inside the cell.

Radioactivity finally appears in the envelope region of the gel only after a 4 min pulse in the pulse-chase experiment. Since NVP63 does not seem to be cleaved during the period of this experiment, the labelled envelope protein must be E1. The simultaneous appearance of NVP97, NVP63 and E1 after a 5 min labelling period in the pulse experiment indicates that the cleavage to give NVP63 and E1 takes place only after completion of their precursor (NVP97).

The sequential appearance of the proteins in the pulse-chase experiment combined with the demonstration of a single site for their synthesis indicates that the order of genes for the structural proteins of the virus is C–E2/E3–E1. It is not possible to order E2 and E3 from the present data since the cleavage to give these two products was not detected. It should be possible to arrange them in the correct order by observation of complete virions released during this type of experiment.

These results show that there is a striking parallel between the synthesis of alphavirus structural proteins and that of picornavirus proteins in that, in both cases, initiation occurs at a single site, the capsid proteins are synthesised first and they are cleaved rapidly from the nascent peptide chain. In the alphavirus system this rapid cleavage may be related to the fact that the remainder of the proteins are destined for glycosylation and incorporation into membranes. The removal of the capsid protein would thus avoid its association with membranes and allow the rapid insertion into nucleocapsid particles observed by Söderlund[22].

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Fig. 2 Infected cells were treated as in Fig. 1 except that after incorporation of ³⁵S-methionine, the radioactive medium was replaced with minimal medium containing 1 mM non-radioactive methionine (pulse–chase experiment), and further incubated for 15 min after restoring isotonicity, when the cell sheets were frozen and processed as in Fig. 1. Cells were labelled for (*a*) 1 min, (*b*) 2 min, (*c*) 3 min, (*d*) 4 min, (*e*) 5 min or (*f*) 6 min after restoration of isotonicity.

Purification and ultrastructure of Aleutian disease virus of mink

ALEUTIAN disease of mink is an immune complex disease induced by viral infection. Major abnormalities include glomerulonephritis, arteritis, plasmacytosis and hypergammaglobulinaemia[1,2]. Infectious virus–antibody complexes have been found in the serum of infected mink[3], and deposits of viral antigen, antibody and complement have been demonstrated in glomerular and arterial lesions[4-6]. Detailed sudy of Aleutian disease virus (ADV) has been hampered by an inability to isolate and purify it by conventional techniques, mainly because ADV obtained from mink tissue is complexed with specific antibody[7]. The immune complexes are not sufficiently homogeneous for purification by physical or chemical properties. Applying techniques that dissociate antigen–antibody complexes to crude preparations of ADV, Cho and Ingram purified viral antigenic material[8,9]. With the electron microscope they observed virus-like particles in both immune precipitates and viral antigen from CsCl gradients. Although the immune precipitates were infectious for mink, lack of quantitative infectivity data on the CsCl gradient fractions prevented identification of these particles as ADV. We have now succeeded in purifying similar virus particles and have identified them as ADV by quantitative infectivity titration.

The Utah I ADV strain[4] (obtained from Dr D. Porter in fourth passage as a 10% liver suspension) was propagated and assayed in sapphire mink. Virus stocks were 10% spleen homogenates prepared from pooled spleens of mink inoculated intraperitoneally 9–10 d previously with 0.5 ml of the previous passage virus stock. Ten per cent spleen homogenates were made in phosphate-buffered saline containing 0.002 M ethylene diamine tetraacetate (PBS–EDTA). Homogenates were sonicated
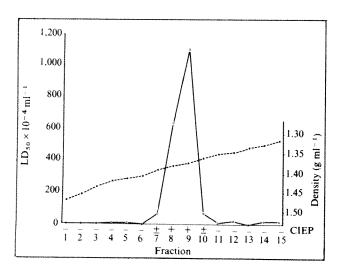
Fig. 2 Electron micrographs of purified ADV pelleted from CsCl gradient fraction 9. Diameter of intact virions is 23 nm. Apparently hollow, ring structured virions are seen in upper left and lower right. Preparations were made with 2% sodium tungstosilicate as negative contrast material.
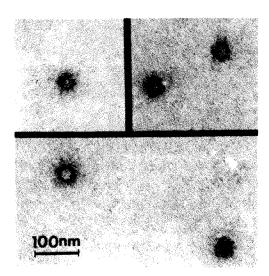
for 60 s in a Biosonik sonicator (Bronwill Scientific, Rochester), clarified by centrifugation at $500g$ for 10 min and stored in small volumes at $-70\,^{\circ}$C. We used the seventh passage of Utah I ADV which had a titre in sapphire mink of 2×10^6 LD_{50} ml^{-1} $(2 \times 10^7$ LD_{50} g^{-1} of spleen). For further purification 10-ml samples of homogenate were thawed, and debris was removed by centrifugation at $500g$ for 5 min and at $6,000g$ for 10 min. The infectious material was pelleted from the clarified suspension by ultracentrifugation at $243,000g$ for 90 min. Pellets were resuspended in 3 ml of PBS–EDTA with the aid of sonication and shaken with 7 ml of ether at room temperature for 5 min to extract lipids from tissue membrane material. The phases were separated by centrifugation at $500g$ for 5 min, and the aqueous phase was mixed with an equal volume of 0.1 M glycine-HCl buffer, pH 2.8. The mixture was incubated for 1 h at room temperature to dissociate antibody–virus immune complexes and ultracentrifuged as before to pellet the ADV and leave eluted antibodies in solution. Pellets were resuspended in PBS–EDTA by sonication, mixed with CsCl to a final density of 1.38 g ml^{-1} and ultracentrifuged for 42 h at $83,000g$ (maximum) at 4 $^{\circ}$C. Fractions were collected through a hole in the bottom of the tube, and the densities were measured by weighing 0.1-ml samples. Fractions were then dialysed against PBS, tested for viral antigen by counter immunoelectrophoresis (CIEP)[7], and stored at $-70\,^{\circ}$C. Infectivity was assayed by intraperitoneal inoculation of two to four sapphire mink with 0.5-ml volumes of serial tenfold dilutions of the dialysed fractions. Mink were tested monthly for hypergammaglobulinaemia and specific anti-ADV antibody. Those that remained clinically and serologically normal for 6 months were considered uninfected. Aleutian disease was confirmed at necropsy in all serologically positive mink.

Fig. 1 CsCl gradient purification of ADV. $\bigcirc$, $LD_{50} \times 10^{-4}$ ml^{-1}; $\bullet$, density (g ml^{-1}). Results of CIEP tests on individual fractions are shown along the abscissa.

Table 1 ADV infectivity titration of CsCl gradient fractions

	Fraction	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	10^{-4}	0/2*	0/2	0/2	1/2	1/2	0/2	2/2	2/2	2/2	2/2	1/2	2/2	1/2	2/2	2/2
Dilution	10^{-5}							2/2	2/2	2/2	2/2	1/2	1/2	0/2	1/2	1/2
Inoculated	10^{-6}							0/2	2/2	4/4	0/2	0/2	0/2	0/2	0/2	0/2
	10^{-7}									1/4						
$LD_{50} \times 10^{-4}$ per 0.5 ml		<1	<1	<1	1	1	<1	32	>320	560	32	3.2	10	1	10	10

Each mink inoculated intraperitoneally with 0.5 ml of dilutions indicated. LD_{50} calculated by Spearman–Karber method. Values plotted as $LD_{50} \times 10^{-4}$ ml^{-1} in Fig. 1.
* Number of mink affected with AD/number of mink inoculated.

A summary of results is shown in Fig. 1, and the detailed data from the infectivity titration are shown in Table 1. Although ADV infectivity was present throughout most of the CsCl gradient, a sharp peak of infectivity was concentrated at a density of 1.37–1.38, corresponding with the ADV antigen peak determined by CIEP. All fractions were ultracentrifuged at 243,000g for 90 min, and the pellets were examined in the electron microscope. Numerous 23-nm particles identical to those seen by Cho and Ingram were found in fractions 8 and 9 only (Fig. 2). Most particles were intact, but some seemed to be hollow as the negative contrast material penetrated the capsid and gave rise to a ring structure. All other fractions were negative for virus in the electron microscope, probably because of the lower ADV infectivity titres seen outside the main peak. It is interesting that the top fraction in all gradients was positive for ADV by CIEP. This could be because either ADV–antibody complexes were incompletely dissociated or non-infectious ADV antigen was found at a low density because of the absence of nucleic acid. No intact or hollow virions were seen in the electron microscope in the top fraction. A large amount of protein debris was, however, present in this region, and this could have masked recognisable virus particles. These results agree with those of Cho and Ingram as to density and morphology of their ADV antigen and provide quantitative infectivity data to indicate that the particles banded in CsCl gradients at a density of 1.37 are indeed those of ADV.

Others[10,11] have reported a buoyant density value of 1.21 for ADV. It is not clear why our value differs from this. In the conditions of centrifugation used by Yoon et al.[11] (30–60% sucrose gradient), the maximum density particle that would band at equilibrium would be 1.28 g ml^{-1}, the density of 60% (w/w) sucrose. Unless the virus particles were extremely large, centrifuging for only 2.5 h at 38,000 r.p.m. would not allow equilibrium to be attained in any case. It seems likely that velocity, rather than equilibrium, centrifugation was performed, and the location of the suspected virus material in the gradient was not an indication of the true buoyant density of the virus. Density values as low as 1.21 could also be explained by the association of lipids with the virus particle. The infectivity of ADV is not, however, affected by extraction with ether. This strongly implies an absence of lipid in the infectious particle.

The size, morphology and density of ADV reported here are consistent with the suggestion that ADV is a member of the parvovirus group (DNA-containing)[1,12]. The type of nucleic acid present in the virion has not been demonstrated. It is likely that with the availability of a simple purification technique this and other basic information on properties of ADV will be readily forthcoming.

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C-type virus antigens detected by immunofluorescence in human bone tumour cultures

IN several animal species, C-type oncogenic RNA viruses (oncornaviruses) cause malignant tumours of mesodermal origin, such as leukaemia, lymphomas and different kinds of sarcoma[1]. C-type oncornaviruses have rarely been observed in human mesenchymal neoplasms using electron microscopy[2,3], but it has been claimed that molecular-biological methods can demonstrate in the tumours the presence of entities, the nucleic acids of which have sequences in common with the genomes of known mammalian oncornaviruses[4-7]. There have also been claims that C-type viruses can be detected in tissue cultures of human tumours[8-10], although it seems that a contamination with an animal virus had occurred in these cases. Recently[11] an agent with type C RNA tumour virus characteristics has been isolated from cultured human acute myelogenous leukaemia cells.

A common cytoplasmic antigen has been repeatedly observed in cultures of human sarcomas with sera from sarcoma patients or their relatives[12-15]. The cross-reactive nature of

Fig. 1 Human chondrosarcoma culture stained with a guinea pig antiserum to the major polypeptide of the SLV at dilution 1:20. For indirect immunofluorescence the microscope slides were sprayed with Teflon, while they were covered with a template, leaving eight wells open for incubation. Five of the wells were filled with different dilutions of the antiviral antiserum. One well was filled with the appropriate normal serum, and two wells with phosphate buffered saline (PBS). The slides were incubated for 30 min at 37 °C in a humid atmosphere, and washed thrice in PBS. After this, seven wells were incubated with the respective FITC-conjugate for 30 min at 37 °C; one well, previously incubated with PBS, was again incubated with the buffer. After washing and drying, the slides were embedded in Elvanol, and screened for granular cytoplasmic fluorescence with a Leitz Orthoplan microscope using Ploem illumination. A CS100 W/2 lamp was used for epi-illumination through a 40/1.0 objective (Zeiss). Narrow-band excitation and emission was performed using the following filter combination: 2 × KP 490 nm, BG 38/4, GG 455 nm, TK 495 nm (interference dividing plate) and SAL 525 nm as barrier filter. Microphotographs were taken with an Orthomat camera on Ilford HP 4 or Agfachrom 50S professional film. Tumour cell cultures originating from fresh tumour material (Dutch Bone Tumour Registry) were maintained in the Pathological Laboratory, Leiden. Control animal cell lines and cells infected with an animal oncornavirus were maintained at the Radiobiological Institute TNO, Rijswijk. To avoid contamination of the human cell lines with an animal virus, no animal cell lines were kept in the Pathological Laboratory, Leiden. For routine electron microscopy the tumour cell cultures were fixed in situ and removed from the bottle by scraping with a rubber spatula. No conspicuous sign of the presence of C-type virus was found in thin sections of these cells. Samples from many subcultures were cultured on microscope slides in Leighton tubes. When confluency was almost reached, the cells were fixed in cold acetone (−20 °C) and after washing in phosphate-buffered saline, stored in a deep freezer.

Table 1 Endpoint titration for immunofluorescence with antisera to mammalian C-type oncornavirus

Cell lines	Rat/RLV No. 45	Rat/RLV No. 76	Guinea-pig/SLV	Goat BoLV
BALB/c embryonic fibroblast (EF)	—	—	—	—
Rhesus monkey kidney	—	—	—	—
Bovine lung embryonic fibroblast (LEF)	NT	NT	—	—
Three human adult fibroblast lines	—	—	—	—
Human embryonic kidney	—	—	—	—
BALB/c EF+RLV	2,560	5,120	80	—
Woolly monkey fibrosarcoma	160	80	1,280	—
Bovine LEF+BoLV	NT	—	—	160
Several human bone tumour lines	20–160	—	40–80	—

Cultures were grown in minimal essential medium (Flow, Irving, Scotland) supplemented with glutamine, non-essential amino acids, antibiotics and 20% foetal bovine serum (Flow). The serum concentration was gradually diminished to 10% after a few days. The cultures were regularly tested for the presence of *Mycoplasma*[19]; all lines have proved to be negative so far. The isoenzyme patterns and the chromosome complements of the tumour lines have been studied. The cells proved to be human and not HeLa[20]. Antisera to RLV were prepared by hyper-immunisation of adult WAG/Rij rats with cells from a syngeneic lymphosarcoma induced by this virus[21]. The antisera were tested by immuno-diffusion and immunofluorescence for their reactivity and specificity. Only those antisera which in immunofluorescence gave a positive fluorescence in BALB/c murine embryonic fibroblasts infected with RLV at a titre of 1:2,560 or higher and no reaction at any serum dilution with uninfected cells were used for the study of human tumour cultures. The antisera were also tested for the interspecific, so-called gs-3 reactivity by immunofluorescence on cat embryonic fibroblasts infected with the feline leukaemia virus[22] or on rat embryonic fibroblasts, treated with 5-bromodeoxyuridine and dimethylsulphoxide for the induction of endogenous C-type virus[23]. All high titre anti-RLV sera were positive in both tests, giving a reaction up to an average endpoint of 1:160. A guinea-pig antiserum to the major polypeptide of the helper virus (SLV) in the woolly monkey sarcoma virus complex[24], was kindly provided by Dr R. V. Gilden (Flow, Rockville, Maryland)[25]. Its specificity was assessed by a strong reaction with a culture from a woolly monkey fibrosarcoma and negative reactions with cultures from tissues of different primate species. An antiserum to the bovine leukosis virus (BoLV) was obtained from a goat with a lymphatic leukaemia induced by neonatal inoculation with BoLV. The antiserum was kindly provided by Professor A. Ressang, Centraal DiergeneeskundigInstituut, Rotterdam. It does not react with uninfected bovine or ovine embryonic fibroblasts, but gives a significant reaction with infected cultures. Fluorescein-isothiocyanate (FITC)-conjugated antisera to the immunoglobulins of various species (Nordic N. V., Tilburg) were first tested for specificity using immunoelectrophoresis. Only those slides in which the wells incubated with normal serum, FITC-conjugate only or phosphate-buffered saline (PBS) were negative, were subjected to further study. A positive and negative control was included in every experiment. They consisted of cells infected with the virus to which an antiserum was directed and the corresponding uninfected cell line.

this antigen has been considered indicative of a virus because different C-type viruses isolated from the same species have strong cross-reactive antigenicity. The C-type oncornaviruses isolated from different mammals usually have some antigenic determinants in common[16-18]. No cross reactivity has, however, been reported between the common human sarcoma antigen and mammalian oncornaviruses. We report that, using immunofluorescence with human bone tumours, we regularly detected antigens which have determinants in common with the Rauscher murine and simian leukaemia viruses (RLV and SLV).

Table 2 Correspondence in positive fluorescence of human bone tumour cultures with anti-RLV and anti-SLV

Human bone tumour cell line	No. of passages	Rat/RLV No. 45	Guinea pig/SLV
Osteosarcoma 1	4	1:160	1:40
	5	1:80	1:40*
	30	—	
	31	—	1:80*
Giant cell tumour 1	9	—	—
4	8	1:80*	1:40*
Chondrosarcoma 1	7	1:20*	1:40*
Chondroblastoma 1	1	1:20*	1:40*
Adamantinoma 1	1	—	—

*Some cells positive.

Using indirect immunofluorescence, cultures were screened for the presence of different cytoplasmic C-type oncornaviral antigens (see legend to Fig. 1). Table 1 shows that the various antisera do not react with uninfected animal cell lines. They give a very strong reaction with cultures infected with the viruses to which the antisera are directed, but there is a significant cross reaction between RLV and SLV. The bovine leukosis virus (BoLV) did not show a cross reaction with either RLV or SLV.

Only one out of six rat antisera to RLV tested so far reacted with several human bone tumour cultures. The negative results with the other high titre antisera excludes a possible contamination with RLV. The fact that these antisera gave a good positive gs-3 reaction is an indication that the human cultures are not infected with the feline leukaemia virus or the endogenous rat virus.

The woolly monkey virus antiserum also gave a positive reaction with several human bone tumour cultures (Fig. 1). In those cases in which the results with the rat anti-RLV serum (No. 45) could be compared with those of SLV, there is a good correspondence (Table 2). As rat anti-RLV serum (number 76) reacts with a SLV-producing culture but not with any human bone tumour culture, it can be deduced that the human cell lines are also not contaminated with SLV. Tumour lines, which reacted with anti-RLV or anti-SLV serum, did not react with the goat serum that specifically reacts with the BoLV. This suggests that the cells are not contaminated with BoLV present possibly in foetal bovine serum. As this antiserum is not very strong, more studies with high titre antisera are needed.

The fluorescence with anti-RLV or anti-SLV varied per passage as shown for two tumour lines in Table 3. Most cells were positive in some passages, in others only a few cells and, in several passages no positive cells could be detected. On continued passage, however, bright fluorescence reoccurred. Our general experience is that especially early passages are highly positive. The positive fluorescence of human bone tumour cultures with patients' sera also varied per passage[13-15]. Positive fluorescence was observed in one or more passages in cultures of two osteosarcomas, four out of five giant cell tumours, one chondroblastoma, two out of four chondrosarcomas. One adamantinoma was negative during several passages.

To test further the specificity of the reaction with human tumour lines, the antisera were absorbed with RLV isolated

Table 3 Variation in positive immunofluorescence of human bone tumour cultures with anti-SLV per passage

Giant cell tumour 4		Osteosarcoma 1		Adult fibroblast 3	
Passage	Fluorescence	Passage	Fluorescence	Passage	Fluorescence
3	+	2	—	2	—
5	—	4	+	5	—
6	—	5	+*	11	—
7	—	11	+*	13	—
8	+*	15	+*	14	—
		20	+	17	—
		30	—		
		31	—		

*Some cells positive.

Table 4 Absorption tests for the specificity of the immunofluorescence reaction of human bone tumour cultures with rat anti-RLV serum No. 45

Absorption procedure	Endpoint titration	
	BALB/c EF + RLV	Human bone tumour cultures
None	2,560	80
In vitro with RLV*	40	—
In vitro with MTV*	640	40
In RLV-infected BALB/c mice†	80	—
In normal BALB/c mice†	640	20

*Antiserum was mixed with an equal volume of virus in buffer (concentration 1 mg ml⁻¹) and stored overnight at 4 °C. Thereafter, the mixture was ultracentrifuged at 100,000*g* for 1 h and then tested in the immunofluorescence test. An additional control was the mixing of antiserum with an equal volume of PBS and subjecting this mixture to the same procedure.

†Antiserum (0.5 ml) was injected into 8 week old BALB/c mice or into BALB/c mice with RLV-induced erythroblastosis of the same age.

from plasma of leukaemic mice[26] or with murine mammary tumour virus isolated from tumours[27]. An *in vivo* absorption experiment was also performed. Both absorption procedures markedly reduced in the immunofluorescence assay on RLV-infected cells and completely blocked the reaction with human tumours (Table 4). The control absorptions only slightly diminished the reaction in both systems, indicating the specificity of the reaction of human tumour cells with anti-serum No. 45. In the same way absorption of the antiserum to SLV with SLV, isolated from tissue culture fluid, blocked the reaction with human tumour cultures completely, whereas absorption with the murine mammary tumour virus had only a slight effect.

The results suggest that cultures from human bone tumours harbour a viral entity, that occasionally produces antigens which have determinants in common with known animal C-type oncornaviruses. It is not likely that the observed reactions result from contamination with murine, feline, bovine viruses or SLV. A more extensive study is needed with a greater variety of antisera to purified proteins from many different animal viruses, to assess the relationship of the putative human virus to the other viruses and to detect whether it is endogenous or exogenous. Also more sensitive techniques than the immunofluorescence method we used are needed to detect viral antigens. Furthermore, tissue culture systems must be developed which lead to a more constant production of antigen and, hopefully, to the production of detectable amounts of C-type particles. Even then, the association of this virus with the causation of bone tumours remains to be established.

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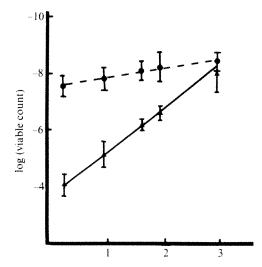
Mechanism of an action of an antibacterial murine lymphokine

THE inactivation and clearing of infectious agents through adaptive immune responses results from interactions between T lymphocytes, B lymphocytes and mononuclear phagocytes[1-4]. The effector cells in cell-mediated immunity (CMI) are primarily T lymphocytes and mononuclear phagocytes[1,5,6]. CMI responses appear in two stages, lymphocyte activation with subsequent production of biologically active mediators, and recruitment and activation of other cells (particularly mononuclear phagocytes) as a result of the activity of several of these mediators[1,3]. Collaboration between T lymphocytes and macrophages in bacterial infections mediated by a lymphokine that causes inhibition of the growth of organisms within the macrophage has been assayed[1,5,8]. This inhibition probably results from increased macrophage microbiocidal activity[1,5]. The subject of this report is a lymphokine that reduces the viability of bacteria. The mechanism of action of this lymphokine is impairment of the ability to divide of otherwise metabolically active bacteria.

The antibacterial activity was assayed on a diaminopimelic-acid (DAP)-dependent auxotrophic mutant of *Escherichia coli*, cultured in DAP-free supportive medium. DAP is utilised in the synthesis of the pentapeptide normally involved in cross-linking of polymeric macromolecules in cell walls and as a source of intermediates essential for lysine synthesis[9]. Incubation in DAP-free medium resulted in formation of spheroplasts and filamentous forms and gradual termination of protein synthesis. Supernatants containing lymphokine were prepared by activation of lymphocytes with a streptococcal filtrate[10] and by the lympho-

Fig. 1 Dose-dependent reduction in viable count of bacteria exposed to the purified lymphokine. The maximum ratio (control :lymphokine-treated) was approximately 2,000:1 at the highest concentration. ●, Control; ▲, lymphokine-treated.

Table 1 Incorporation of labelled metabolites into bacterial cell macromolecules and corresponding viable counts in cultures exposed to purified lymphokine and control preparations

Metabolite and isotope	Lymphokine (c.p.m.)		Control (c.p.m.)	
Incorporation in deprived cultures (3 h pulse)	(expt. 1)	(expt. 2)	(expt. 1)	(expt. 2)
^{14}C-amino acids	961 ± 17	63 ± 28	564 ± 308	1,200 ± 937
^{3}H-uridine	25,667 ± 3,700	1,081 ± 177	172,800 ± 91,700	94,600 ± 31,800
^{3}H-thymidine	3,690 ± 1,480	102 ± 30	2,180 ± 420	1,174 ± 410
Incorporation during recovery (24 h pulse)				
^{14}C-amino acids	39,430 ± 6,610	42,080 ± 9,460	54,900 ± 16,800	51,750 ± 1,970
^{3}H-uridine	200,400 ± 47,200	128,370 ± 28,960	338,700 ± 2,210	135,330 ± 32,800
^{3}H-thymidine	5,910 ± 1,290	62.0 ± 18.0	19,220 ± 2,210	4,060 ± 1,180
DAP-dependent viable count	7.8 ± 1.2 × 10^7	5.9 ± 0.8 × 10^4	5.2 ± 1.5 × 10^8	1.01 ± 0.6 × 10^8
NON-DAP-dependent viable count (Colonies on TSA without DAP)	9.1 ± 0.8 × 10^5	0	6.0 ± 0.8 × 10^4	1.40 ± 0.9 × 10^2

Bacteria were suspended at a density of 10^9 organisms ml^{-1} in DAP-free supportive medium containing lymphokine or control preparations and incubated for 10 d. At the end of this period, 0.5 ml of culture suspensions were added to 0.5 ml of medium containing 0.1 μCi of labelled tracer (Amersham), and incubated for 3 h. At the end of the incubation, culture suspensions were diluted 1:10 in supportive medium containing DAP, and 0.5 ml of this incubated with tracer as above for 24 h to assay recovery. Viable counts were performed on DAP-containing Tryptose Soy Agar (TSA) plates. Processing and determination of incorporation of label was after the method of Bartholomaeus *et al.*[12]. To optimise incorporation of ^{3}H-thymidine, 250 μg ml^{-1} deoxyadenosine was added with the isotope as an inhibitor of cytoplasmic thymidine kinase[7].

cyte phytohaemagglutanin (PHA)-coated L-cell coculture method[11]. Lymphocyte preparations (greater than 95% non-phagocytic small mononuclear cells) consisted of spleen lymphocytes prepared on Ficol–Isopaque gradients[12] mixed with equal numbers of thymus lymphocytes. Lymphokine-containing and control supernatants were concentrated on a UM 10 membrane in an Amicon Diaflo apparatus. The lymphokine was purified by Sephadex G-200 gel filtration and Pevicon block electrophoresis.

The purified lymphokine was trypsin-sensitive and gave a single band on analytical acrylamide gel electrophoresis and on immunoelectrophoresis. No cross reaction with an antiserum to foetal bovine serum could be detected. The lymphokine had a molecular weight (from Sephadex gel filtration) of 75,000 ± 15,000 and electrophoretic mobility similar to that of a fast α globulin. The lymphokine produced in lymphocyte–PHA–L-cell cocultures could not be differentiated from the lymphokine produced by stimulation of lymphocytes with streptococcal filtrates.

In cultures exposed to the lymphokine there is a reduction in viable count differing from cultures exposed to control preparations by factors up to 2,000 (Fig. 1). Exposure of wild-type *E. coli* to the lymphokine throughout a growth cycle produced an increased rate of loss of viability in stationary phase (R.L.P.F. K.J.T., and M.P.A., unpublished).

In the assay utilising the DAP-dependent mutant both lymphokine-treated and control cultures contain metabolically active organisms capable of incorporating similar amounts of labelled amino acids into protein, and uridine into RNA in recovery of metabolic activity when provided with limiting nutrient after deprivation (Table 1). The drop in viable count in lymphokine-treated cultures correlates with the almost total loss of the capacity to incorporate ^{3}H-thymidine into DNA in cultures which remain DAP-dependent (Table 1). These results are consistent with the hypothesis that the primary effect of the lymphokine is impairment of bacterial nucleic acid metabolism expressed in the inhibition of the synthesis of new DNA, and thus, loss of the ability to divide and consequent drop in viable count. In deprived cultures the effect extends to depression of RNA metabolism. This suggests that transcription as well as DNA replication may be inhibited.

The relationship between this lymphokine and the factor that inhibits the growth of microorganisms detected in the serum of immune mice after challenge with antigen[13] is of considerable interest. Investigation of the relation between these factors will give a clear indication of any involvement of lymphokines in direct antibacterial immune responses. Nonspecific production

of lymphokines in response to supernatants from streptococcal cultures would suggest that some nonspecific responses to bacteria[14] may be mediated similarly by lymphokines.

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Corrigenda

In the article "Origin of fifteen cosmic dust particles intercepted by Pioneer 8 and 9" by J. W. Rhee, O. E. Berg, F. F. Richardson and S. Auer (*Nature*, **252**, 555; 1974), the second sentence should read "The two spacecraft are in direct heliocentric orbits with perihelia of 0.99 AU and 0.75 AU and aphelia of 1.09 AU and 0.99 AU, respectively."

In the article "Re-interpretation of Devensian Till stratigraphy of eastern England" by P. A. Madgett (*Nature*, **253**, 105; 1975), there was an error in Fig. 2. The abscissa should read

$$\frac{\text{Epidote}}{\text{Epidote} + \text{Zircon}} \times 100$$

and not as printed.

Erratum

In the article by J. de Leiris, L. H. Opie and W. F. Lubbe (*Nature*, **253**, 746; 1975), the title should read "Effects of free fatty acid and glucose on enzyme release in experimental myocardial infarction".

matters arising

Distance to Cygnus X-1

CHENG *et al.*[1] have recently re-examined our estimates[2,3] of the distance to Cygnus X-1. They have noted that the Cepheid V547 Cyg is at $d = 6.6$ kpc, yet has colour excess 1.1, far less than predicted by a uniform reddening extrapolation. On this basis they conclude 'the only independent distance check (on Cyg X-1) is so widely in error.' It is well known (see refs 4, 5) that the dust responsible for interstellar reddening is stratified in layers, and thus for every non-zero galactic latitude there exists a distance beyond which stars show no further colour excess regardless of modulus. In such fields one therefore expects the most distant observable stars to have colour excess no greater than that of intermediate distance objects; V547 Cyg is entirely consistent with this concept. It is also wrong to state that there exist no other independent estimates of distance, as we pointed out previously. Evidence based on spectroscopic modulus[6], linear polarisation[7], cluster membership[8] and interstellar lines[9] all indicate $d > 2$ kpc. Each of these methods has its own assumptions which may be individually questioned and evaluated; none of the methods, however, uses the same assumptions as the colour excess method we used. We find it quite convincing that five independent methods, although none individually of extreme accuracy, are all in agreement.

Cheng *et al.*[1] speculate that the "possibility" of X-ray heating of the primary makes distance estimates uncertain. The amount of heating is a directly measured quantity and has been shown to be entirely trivial[10]. They also state that an extrapolation of the reddening diagram to beyond 1 pkc is unwarranted. But if one adopts $d \leqslant 1$ kpc as they claim as a possibility, then no extrapolation is necessary, and one must then postulate that this star is situated behind interstellar matter which creates reddening per unit distance far exceeding all other of the ≈ 125 objects we measured within 50' of Cyg X-1. The alternative of circumstellar rather than interstellar reddening is excluded by the lack of infrared excess in HDE226868. Furthermore, the polarisation agreement[7] means that this excess material, implied by the authors to be in a small globule, manages in a fraction of a pc to exactly compensate for the linear polarisation otherwise provided by 2 kpc of interstellar matter.

Although we agree that Cyg X-1 is not proven to be a black hole, and other models for the system are still viable, we think that the overwhelming weight of observational evidence places this object at $d > 2$ kpc regardless of its nature.

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Indirect methods for detecting γ-ray bursts from supernovae

PORTER[1] suggests the possibility of detecting γ quanta from supernova bursts with a detector located at sea level. He bases his calculations on the results of a Monte Carlo computation procedure for electromagnetic showers[2]. It follows from the tables of cascade functions[2] that the mean particle number behind the cascade curve peak decreases with depth t as $e^{-\lambda t}$, where λ is much smaller than $\lambda_{\min}$ the minimum cross section of photon absorption in the matter. This result is at variance with the assertion that the shower development at low levels is determined by absorption of photons with the greatest permeability[3]. The experimental data used by Messel and Crawford[2] produced a discrepancy for photon absorption in air compared with the calculations of Ivanenko[4], which they explained by the fact that the Compton scattering had been inaccurately included in his work. Since all essential processes are accurately included in the Monte Carlo procedure, the disagreement with the cascade curves obtained by the momentum method[5,6] was ascribed[2] to

the low accuracy of the reconstruction of the function based on a limited number of momenta

Numerical integration of the cascade theory one-dimensional equations[7,8] makes it possible to include accurately all processes and to calculate the longitudinal shower development down to

Table 1 1 MeV photon absorption coefficient

Element	$\lambda_{\min}$	λ_{calc}	λ_{MC}
Air	0.61	—	0.04 (ref. 2)
C	0.60	0.53 ± 0.01	—
Fe	0.41	0.33 ± 0.01	—
Cu	0.40	—	0.38 ± 0.02 (ref. 11)
			0.11 (ref. 2)
			0.24 ± 0.02 (ref. 4)
Pb	0.27	0.21 ± 0.01	0.25 ± 0.02 (ref. 12)
			0.27 ± 0.03 (ref. 13)

sufficiently low depths to produce a good estimate of the absorption coefficient λ. Inclusion of annihilation of positrons would result in an appreciable increase of the calculation volume because the two-equation system must be replaced by a three-equation system The contribution from annihilation was, therefore, estimated The terms, including particle redistribution resulting from annihilation, were inserted into the system of two transport equations, and the positron number was taken as 0.4 and 0.5 times the total charged-particle number in two separate runs. The calculations were carried out for an electromagnetic shower in lead where the annihilation effect is greatest. Annihilation γ quanta from stopped positrons were also included. The estimate shows that the contribution from annihilation may be neglected to an accuracy of 3–5%. This result is in line with other calculations[9,10].

A lower boundary for λ can be obtained when numerically integrating the one-dimensional equations, since the inclusion of scattering will only increase the cascade particle absorption with depth. For $E > 10$ MeV in heavy elements $E > 1$ MeV in light elements, the scattering effect on the cascade curve shape may be neglected[5,6]. We have calculated the cascade curves in lead, iron, and carbon from a primary (electron or photon) with energy E_0 in the 1–10^3 GeV range for several values of E from 316 MeV to 10 KeV. The calculated and

Monte Carlo data on the $E > 1$ MeV photon absorption coefficient λ (refs 4, 11–13; Table 1) are in agreement. The difference of the calculated and Monte Carlo λ from λ_{min} can be explained by the effects of Compton scattering and multiple Coulomb scattering.

Comparison between the cascade curves for the various threshold energies has shown that the data tabulated in ref. 2
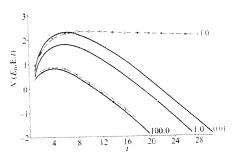
Fig. 1 The mean number of photons, $N (E_0, E, t)$ with energies exceeding E from a primary photon with $E_0 = 10$ GeV in carbon at depth t. The solid curves show the results of numerical integration, the dashed curves represent the data from ref. 2. The numerals on the curves indicate the threshold energy E in MeV.

agree with the results obtained using the numerical integration and momentum method[5,6] for $E > 10$ MeV in lead and $E > 100$ MeV in air. Figure 1 shows the typical cascade curves from Messel and Crawford's tables for air and those calculated in the present work. The results obtained cast doubt on the conclusion drawn by Porter[1] about the possibility to detect the bursts from supervonae with a mountain or sea-level detector, since the $E > 1$ MeV photon number in ref. 2 is overestimated by a factor of 10^2 at mountain level and by a factor of about 10^4 at sea level.

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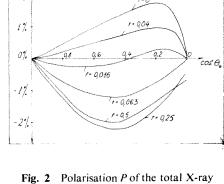
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Solar flare X-ray polarisation

BROWN *et al.*[1] have argued that the "Intercosmos-1" and "Intercosmos-4" results of solar flare X-ray polarisation measurements are in error due to incorrect 'normalisation' procedure. The normalisation was made in order to eliminate the effect of possible differences in the sensitivity of the photon counters and it is based on the assumption that the X-ray flux is unpolarised during the decay stage of flare[2].

According to ref. 1 the Thompson scattering of intrinsically isotropic and unpolarised flare emission taking place in the lower parts of the solar atmosphere could give polarisation P up to some 30% of observed X-ray flux (the sum of primary and scattered radiation). This conclusion is based on consideration of the scattering at 90°. In fact, because of weak dependence of the Thompson cross section on the scattering angle χ the observed flux corresponds to a wide range of χ (Fig. 1). Integration over all possible χ gives a rather low value of P (ref. 3). The area of the photosphere just under the flare gives scattered flux polarised mainly in trans-

Fig. 1 Scattering of the flare X rays by the solar photosphere.

verse direction ($P < 0$), while the flux from more remote areas has a preferentially radial electric vector ($P > 0$). The resulting value of P depends on the altitude of the flare $\tau = H/R_\odot$ and on the heliocentric angle θ (Fig. 1).

Detailed calculations of the back scattered radiation in the first order scattering approximation are given in ref. 3. For the total flare X-ray flux we always have $|P| \leqslant 2\%$ (ref. 3 and see Fig. 2). Multiple scattering could alter this value by as much as a factor of two (ref. 3). It was taken into account in the computation of solar X-ray albedo using the Monte Carlo method[4], but the

Fig. 2 Polarisation P of the total X-ray flux as function of heliocentric angle θ.

statistical significance achieved was too poor to determine the polarisation.

So inclusion of Thompson scattering effects cannot substantially influence the results of normalisation in ref. 2.

In further experiments[5] the scatterer and the counters were mounted on a rotating drum. This permits continuous checking of the relative sensitivities and thus eliminates the need for normalisation. Unfortunately, we were not able to obtain absolute values of P with the polarimeter on board "Intercosmos-7" because of the failure of one of its measuring channels. The polarimeter of "Intercosmos-11" has worked well and results obtained will be published elsewhere.

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Are centromeric dots kinetochores?

EIBERG[1] described a Giemsa staining technique which reveals specific paired dots in the centromere region of human chromosomes in cells treated with colcemid and hypotonic KCl. He suggested that the dots may represent organelles associated with spindle fibres. Evans and Ross[2] demonstrated similar, stained or unstained dots obtained in colcemid-treated mammalian cells by other preparatory techniques. They hypothesised that these dots "may represent the kinetochores and particularly their associated proteins".

These interpretations are unlikely for several reasons. First, the location of the unstained dots shown by Evans and Ross[2] does not coincide with that of the raised dots on unstained, shadowed chromosomes. Unstained dots adjoin chromosomes in the centromere region, whereas raised dots, like Eiberg's[1] C_d dots, lie clearly within the chromosomes. Second, there is no evidence that the centromerically located spindle microtubules are always present in colcemid-treated cells. A two hour exposure of rat kangaroo cells to 0.05 µg ml^{-1} colcemid destroys all microtubules and alters the fine structure of the kinetochores[3]. Speculation that spindle proteins concentrated in the centromere regions of chromosomes could produce centromeric dots cannot, therefore, be substantiated. Third, kinetochores are rather delicate, labile organelles and they are probably affected drastically by treatments used in the preparation of chromosomes for light microscopy. For example, no kinetochores are visible in thin sections of Chinese hamster chromosomes subjected to the aceto-orcein smear technique (L. J. Journey, personal communication). Fourth, even if kinetochores were preserved by light microscopic techniques they could hardly produce prominent raised dots, for the disk-shaped kinetochores of mammalian chromosomes are only some 100 nm thick[3,4].

Optical and electron microscopic observations of rat kangaroo cells treated with hypotonic colcemid[5] suggest a different interpretation of centromeric dots. Paired dots are recognisable by phase contrast microscopy in the centromere region of favourably oriented chromosomes in embedded prometaphase cells (Fig. 1a). In thin sections the dots appear as patches of chromatin packed more densely than in the remainder of the chromosomes (Fig. 1b). The kinetochores which are less opaque to electrons lie adjacent to these patches. In metaphase cells the chromosomes are, overall, more condensed, but the chromatin is also more densely packed in the centromere region than in the arms (Fig. 1c). The outer layer of each kine-
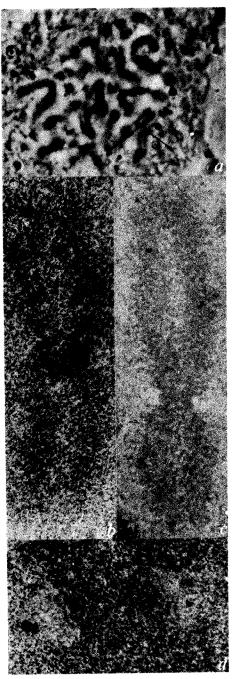
Fig. 1 Chromosomes of rat kangaroo cells (line PtK$_2$) treated with hypotonic colcemid (15 min at 37 C in 0.25 µg ml^{-1} colcemid prepared by diluting an aqueous 0.5 µg ml^{-1} stock solution 1:1 with culture medium. See ref. 3 for culture conditions, fixation and embedding). *a*, Phase contrast micrograph of a prometaphase cell (× 1,625). Note the paired dots in the centromere region of favourably oriented chromosomes. Single dots are visible on laterally viewed chromosomes. *b*, Thin section of the arrowed chromosome of Fig. 1a × 11,700. Note the two patches of densely packed chromatin in the centromere region. *c*, Metaphase chromosome × 7,475. Note the dense centromeric chromatin and the adjoining electron-lucent zones. *d*, Centromere region of another metaphase chromosome × 22,425. Note the kinetochore bands at the surface of the primary constriction and the electron-lucent zones.

tochore appears as a moderately electron-opaque band lying parallel to the chromosomal surface in a distinctly electron-lucent, approximately circular zone adjacent to the centromere region (Fig. 1c and *d*).

These electron-lucent zones correlate with, and may be equivalent to, the unstained dots described by Evans and Ross[2]. This interpretation is supported by Journey's observations (personal communication) of "empty", circular zones adjacent to the centromere region of Chinese hamster chromosomes in thin sections of acetoorcein smears. The densely packed centromeric chromatin, on the other hand, may account for the dots revealed by Eiberg's[1] Giemsa staining technique and by the shadowing technique of Evans and Ross[2]. Dense structures reminiscent of centromeric dots are also seen in the centromere region in whole-mount preparations of Chinese hamster chromosomes treated with distilled water[6] and in whole mounts of HeLa chromosomes stained with phosphotungstic acid[7]. All these results can be explained by a greater resistance of the centromeric chromatin to the spreading forces to which chromosomes are subjected in a hypotonic medium. This resistance may reflect a specific DNA-protein composition of the centromeric chromatin, that in turn could account for the specific staining, as pointed out by Eiberg[1].

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Logic of animal conflict

MAYNARD-SMITH and Price have shown[1] that it is not necessary to invoke group selection to explain the occurrence of 'conventional' rather than 'dangerous' tactics in animal conflict. The conditions for the evolution, under individual selection, of populations in which conventional tactics predominate are, however, more stringent than they suggest. In particular, given their pay-off matrix, the population may end up consisting mainly of individuals adopting the 'dangerous' strategies, Hawk and Bully.

Consider a population consisting almost entirely of Hawk and Bully, so that nearly all conflicts are between these. Then

Hawk is the better strategy when the opponent is Bully and Bully is better when the opponent is Hawk. Thus we have a system of frequency-dependent selection, leading to a stable mixture of Hawk and Bully. At this equilibrium, Hawk and Bully will have equal fitness on average and will therefore have frequencies: Hawk 0.575652; Bully 0.424348. In nearly all conflicts, the opponent will be Hawk or Bully, with frequencies just given, so that average pay-offs will be: Mouse 19.5000; Hawk 20.4311; Bully 20.4311; Retaliator 11.3932; Prober-Retaliator 13.6357.

Therefore, types other than Hawk and Bully are at a disadvantage and will not spread. These results will not be affected by the presence of a few individuals adopting strategy Mouse for non-genetic reasons, since Hawk and Bully are the best (equally good) strategies when the opponent is Mouse.

If, for simplicity, we regard the five strategies as reproducing asexually with fitnesses given by average weighted pay-offs, we find that the Hawk-Bully equilibrium is attained from some starting points, for example, with strategies given in the order above, (0.33, 0.33, 0.33, 0.005, 0.005) or (0.9, 0.025, 0.025, 0.025, 0.025). On the other hand, starting with all strategies of equal frequency, the ultimate population consist entirely of Retaliator. These results suggest strongly that some modification of the original model is required to explain the general occurrence of conventional strategies.

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Professor Maynard-Smith replies—I am afraid that Gale and Eaves are quite right. They have found an alternative evolutionary stable strategy to the conflict which the late Dr Price and I investigated.

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Anti-Darwinism among the molecular biologists

Ohta[1] asserted that evolutionary change at the macromolecular level was caused primarily by "mutation pressure" rather than Darwinian selection. This view, upheld by an influential school of biochemists and molecular biologists (most of them named in Ohta's bibliography), strikes some of us as regressive and potentially dangerous to our science.

One major danger is that it will dissuade biochemists from looking for functional significances in sequence differences (for example those of cytochrome *c* from different species). There is also a more general danger, that of encouraging ahistorical, statistical and mathematical thinking at the expense of the search for causal and historical explanations of the particularities of organisms.

It was a Scottish philosopher of science who wrote that "Only in the last resource, when the heavenly powers fail us, should resort be had to the demons of the underworld, chance and probability"[2]. In the case of macromolecular evolution, it does not seem to me that Ohta and those who think like him in any way have demonstrated the failure of the "heavenly powers" of Darwinian selection. Earlier, Kimura and Ohta[3] had coined the phrase "naive pan-selectionism" for the ideas of those who disagreed with them, and this phrase was taken up by Wills[4] in a paper, not cited in Ohta's bibliography, which criticised in some detail the arguments of Kimura and Ohta, and showed that the evidence was quite consistent with a selective origin for most, if not quite all, the protein differences met with in nature.

The major argument cited by the followers of Ohta and Kimura in favour of their attitude has been the alleged time-proportionality in the numbers of residues differing between species, for cytochrome *c* and other proteins. Reasons have been put forward, such as the "Red Queen hypothesis" of van Valen[13], why a loose proportionality of this sort might be expected on the basis of ordinary selectionist theory. Ohta, however, treats it as an established fact that there exists a proportionality far too accurate to be explained in this way. It needs to be pointed out that, in the graphs published to illustrate this proportionality, by Dickerson[5] for cytochrome *c*, for example, and by Wilson *et al.*[6] for haemoglobins, the apparent linearity of the relationships depends on the assignment of some very questionable ancestral ages for the taxa concerned. Thus Dickerson gives a mid-Cretaceous age (around 100 Myr) for the ancestries of the main orders of placental mammals, whereas fossil evidence[7] would place this in the very late Cretaceous or early Palaeocene, perhaps 75 Myr ago. For a common ancestry of mammals and reptiles, that is an ancestral amniote, Dickerson cites a Lower Carboniferous (around 320 Myr) age, whereas fossil evidence[7] would suggest an early Permian age of perhaps 270 Myr. For a common ancestor of Amphibia and Amniota, Dickerson's graph assigns a late Devonian age of some 350 Myr, whereas fossil evidence[7] would rather suggest the mid-Carboniferous, some 300 Myr ago. Finally, the age given by Dickerson for a common ancestor of insects and vertebrates is late Pre-

cambrian, perhaps 600 Myr old. This would be about the age of the celebrated Ediacara fauna of Australia, described by Glaessner[8] in which quite advanced annelid and possible primitive types of arthropods are represented; undoubtedly, any common ancestry of the deuterostomian line (including Vertebrata) and the molluscan-annelid one from which the insects sprang must have been very considerably older, perhaps more than 700 Myr old.

The similar graph for the haemoglobins given by Wilson *et al.*[6] assigns some even more questionable ancestral dates—for example, original separation of the cyclostome (lamprey) line from that of Gnathostomata, is placed in the Proterozoic, some 800 Myr ago, probably nearly as old as the entire metazoan line!

A further assumption of Ohta is that protein polymorphism in natural populations is non-adaptive and a result of mutation pressure. Where such polymorphism has been studied in detail for particular proteins, as pointed out by Johnson[12], the phenomena have been found to parallel closely those of polymorphism in ordinary phenotypic characters, selective control of which has been demonstrated, as pointed out by Ford[11] in another important and relevant work not mentioned in Ohta's bibliography. Selander and Kaufman[9], also not cited, draw attention to a book by Levins[10] in which the theory is developed of the long term adaptive advantages of maintaining certain critical degrees (varying with the characters and the circumstances) of heterozygosity of natural populations.

Ohta's evident pride in the mathematical sophistication of his methods of analysis prompts a scriptural gloss on his phrase "naive pan-selectionism": namely, that by adopting what he would consider as the naivety of "little children", the molecular biologists might improve their chances of entering into that "kingdom of heaven" in which the historic truths of evolution are revealed.

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reviews

As a result of intensive research since the beginning of the present century, the traditional image of the Middle Ages as an epoch of sterile subservience to the authority of the Church and of Aristotle in scientific matters has been destroyed. Instead, it is now recognised as a period during which scholars became capable of wide ranging and subtle, albeit habitually inconclusive, speculation upon topics commonly supposed to be the distinctive concern of early modern science—the rotation of the Earth, for example, the atomic structure of matter, the possibility of creating a vacuum, and so on. It has emerged, moreover, that such medi-aeval speculations, although cast in a very different mould, are by no means isolated from the articulation of the new philosophy in the 17th century. On the contrary, as Edward Grant clearly illustrates in *A Scource Book in Medieval Science*, scholastic discussions exercised a patent or possible influence on such later innovators as Copernicus, Galileo and Otto von Guericke. At the other chronological extreme, Profes-sor Grant begins his anthology of mediaeval science with extracts from the late-classical encyclopaedias that preserved an inkling of ancient cul-ture in the Latin west during the Dark Ages, and proceeds thence to illustrate in considerable detail subsequent developments in logic, mathematics, physics, cosmology, chemistry, geo-graphy, biology and medicine. Although the bulk of the material is drawn from the period 1200–1500 AD to have documented approximately a millenium of, at times, intense intellectual activity

Medieval record

C. J. T. Lewis

A Source Book in Medieval Science. Edited by Edward Grant. Pp. xviii + 864. (Harvard University Press : Cam-bridge, Massachusetts, August 1974.) $32.50.

over such a wide range of subjects is obviously no mean achievement.

It is not only the sheer bulk of avail-able material that renders the con-struction of an anthology of mediaeval science so problematic a task, however. In addition, there is the difficulty of selecting topics and texts that are relevant both to the subsequent evolu-tion of early modern science and to the interests of the ordinary 'educated reader' at whom this work is partly directed, without simultaneously dis-torting the emphasis of the original speculations. Professor Grant is, of course, well aware of, and for the most part effectively reconciles, such conflicting demands.

Nevertheless, it is perhaps inevitable that he should tend towards the use of an essentially positivist criterion in making his selections. This tendency is most conspicuous in the devotion of relatively little space to alchemy and astrology, and the complete exclusion of the more exotic magical sciences. More subtly, however, the same criterion determines a concentration on the 'achievements' of mediaeval science

that I have already mentioned. Occa-sionally, this leads to a measure of dis-tortion, most obviously, for example, in the documentation of mediaeval 'kine-matics'.

On the whole, however, such mis-representation is avoided; the broader context of individual problems, the characteristic conceptual interrelation-ships and the typical methods of mediaeval science are illustrated with considerable force and economy. In-deed, a particular virtue of this source book is that the (to a modern mind) metaphysical preoccupation, and the unique gothic complexity, of much scholastic scientific discussion is clearly illustrated by the reproduction of com-plete *quaestiones* and treatises.

It is inevitable in a work of such size and scope that one should take exception to certain features and regi-ster certain omissions. Thus, although the copious annotation of individual documents renders them perfectly comprehensible, it is regrettable that the sections on physics should be pro-vided with neither a contemporary nor a modern outline of the principles of Aristotelian natural philosophy, which constituted, after all, the foundation of most scholastic physical speculation. Again, it seems a pity, considering the possible audience of this book, that further references for given topics are not more thoroughly recorded. Ulti-mately, however, it must be said that this book offers a clear and scholarly exposition of a mass of fascinating documents, and provides an unparal-leled insight into the processes and pre-occupations of mediaeval science. □

Most students find mathematical physics a difficult subject. They can follow a qualitative explanation (such as, "magnetisation lines up the dipoles"), and they can follow the steps in an algegbraic deduction, or in the solution of an equation and the evalu-ation of an integral; the physics and the mathematics are philosophically distinct activities. Difficulties arise, how-ever, at the meeting-point between these disciplines. How does one mathematise a physical problem? Why do the same equations crop up over and over again, in apparently unrelated physical con-texts?

This original undergraduate text is a largely successful attempt to answer the second of these questions. The philosophy is to apply the field equa-

tions of mathematical physics (Laplace, Poisson, wave, and diffusion) to pro-blems of increasing complexity, illus-trating the solutions obtained with examples from a very wide range of

Maths and physics

Michael Berry

Introductory Eigenphysics: An Ap-proach to the Theory of Fields. By C. A. Croxton. Pp. x + 275. (Wiley: London and New York, November 1974.) £6.95 board; £3.95 paper.

fields of physics. First, the field equa-tions are introduced. Then they are solved in rectangular, cylindrical and spherical coordinates. Finally, approxi-mate methods (perturbation, WKBJ and variational) are discussed. The origin-

ality lies in the examples chosen which are oriented towards physics rather than applied mathematics and which include lattice vibrations, Bloch waves, skin effect, aerofoil theory, vibration of a rotating star, earthquakes in the Mohorovicic layer, pseudopotentials in liquid metals, and quantum chemistry. There are many interesting exercises for the reader.

Unfortunately, the book is seriously marred by many minor errors and in-felicitudes, most of which could have been avoided by careful proof-reading. Symbols are not defined, figures are im-perfectly labelled and terms are intro-duced without explanation. This will confuse all but the best students. I do, however, warmly recommend the book to teachers. □

Agriculture and industrialisation

Agriculture and the Industrial Revolution. By E. L. Jones. Pp. xiii+233. (Blackwell: Oxford, February 1975.) £5.50.

As the title suggests, this is a book about the relationships between the development of agriculture in Britain and the process of industrialisation which we refer to as the Industrial Revolution. The work, a collection of essays published by the author during the 1960s, is divided into two main sections. A group of essays concerned mainly with agricultural change and the process of industrialisation constitutes the first part, and a further group round the theme of agriculture in the urban industrial economy comprises the second. Many of the essays are detailed studies of agricultural changes in specific locations, as, for example, are the pieces on farming in Hampshire and Herefordshire. But the author is able to bring a considerable degree of theoretical clarification to his materials. This is nowhere more clearly exemplified than in the essay entitled "Agriculture and Economic Growth in England 1650–1815: Economic Change". In this essay the author takes up vigorously the problem of explaining the essential relationships between agriculture and the Industrial Revolution. In particular he has tried to separate, for the purpose of analysis, the "longer term causes of agricultural change in the centuries before the Industrial Revolution, and agriculture's role in the Industrial Revolution through such activities as capital formation, the release of productive factors and demand expansion". The author concludes that, on balance, the agricultural sector made a valuable net contribution to economic growth during the period 1650–1815. He confirms that the typical textbook argument, for example, that labour was ejected into factory industry by the force of the parliamentary movement, needs closer scrutiny.

There is no space in a review as short as this to venture into the rich detail of the essays nor is there space even to give a brief summary of each one. It may, perhaps, serve to draw attention to the contemporary importance of this volume, if I select a passage from R. M. Hartwell's introduction and indicate the range of questions that the author treats. Did the agricultural revolution produce an industrial revolution or *vice versa*? Did more people produce more food or more food, more people? Did an agricultural surplus allow the growth of industry or did industrial change induce agricultural change? What was the relationship of the agricultural revolution to the Industrial Revolution and to the growth of population?

The use of the past tense in the forming of questions should not, however, obscure their contemporary relevance. Three quarters of the world is trying to achieve modernisation through some combination of agricultural and industrial inputs. Further, the balance chosen as a matter of policy is not infrequently frustrated by a seemingly uncontrollable population expansion. Though no one expects that in the modernisation of the Third World history will (or must) repeat itself, intellectuals and the more practically oriented alike should peruse the pages of this volume if they seek to understand the fine structure of the contemporary problem they are attempting to solve.

Michael Gibbons

Human problems

Ethical, Social and Legal Dimensions of Screening for Human Genetic Disease. Edited by Daniel Bergsma. Pp. viii+272. (Symposia Specialists: Miami, 1974. Distributed by Stratton International Medical Book Co., New York.) $13.95.

THE rise of interest in genetic screening over the last few years has probably been very largely the result of technological developments. As Tabitha Powledge says in her contribution to this work by members of the Genetics Group of the Hastings Institute of Society, Ethics and Life Sciences, "It is regrettably true that, just because we *can* do something, we very often proceed to do it, without thinking much about whether we *should*". A recurring theme throughout this book is that we should have a very clear idea of why we want to screen, quite apart from whether or not it is practical. According to Powledge, and her sentiments are echoed by other contributors, research is a rational and legitimate purpose of screening but in that case screening should not be presented as having a service or of being of direct benefit to the screened.

There are, perhaps, two important reasons for screening for genetic disease. First, if there is an effective treatment which, instituted early enough, could prevent the development of severe mental handicap, for example, in phenylketonuria. Second, so that genetic counselling and, where possible, advice on antenatal diagnosis can be given early enough to prevent the possible birth of another affected child in the family. In initiating such screening programmes it is, however, important to keep in mind the general principles of Wilson and Jungner (*Principles and Practice of Screening for Disease;* World Health Organisation: Geneva, 1968), in particular the provisos that the disorder must be an important health problem, it must be treatable (and/or preventable), the screening test must be reliable, and it must be cost-effective to screen.

In the light of these sentiments the value, and problem, of screening for unifactorial, multifactorial and chromosomal disorders are discussed. In the case of unifactorial disorders the main problem is that most are rare, and even for those that are relatively common a reliable screening test is often not yet available; for example, in fibrocystic disease. Two relatively common disorders which have been considered recently for screening are certain protease inhibitor genotypes and familial hyperbetalipoproteinaemia.

In so-called multifactorial disorders (for example, essential hypertension and diabettes mellitus) the main problem is that even if one had a reliable test for detecting preclinical cases, there is as yet little evidence to suggest that treatment at this stage would prevent the development of the disease. According to Mellman the screening of newborn populations for chromosomal abnormalities is now of doubtful justification, for apart from providing incidence figures it tells us nothing of the natural history of such disorders. The process of identifying individuals in newborn surveys and studying them from birth until social maturity is too slow to satisfy society's impatience for information on say the XYY and XXX phenotypes. Such screening would be more informative if it included school children and even samples of the adult population.

Quite apart from the scientific merit and cost-effective benefit of screening, there are the social, psychological, ethical and legal problems associated with genetic screening. Predictably, participants who volunteer for screening programmes (as in the case of Tay Sachs disease) are younger and, on average, better educated than those who do not wish to participate in such programmes. And certainly the knowledge that one is a carrier of a genetic disease may have profound effects on marriage plans and marital relationships. The legal and ethical problems of whether or not information obtained from screening programmes should be communicated to the person screened, and possibly also to his relatives if medically or genetically indicated, require the most careful consideration.

Issues raised by genetic screening are becoming increasingly important to us all. This collection of essays goes a considerable way towards making us stop to consider the implications.

A. E. H. Emery

Reflections on surfaces

Science and Technology of Surface Coating. Edited by B. N. Chapman and J. C. Anderson. Pp. xvii + 463. (Academic: London and New York, 1974.) £14.80; $41.50.
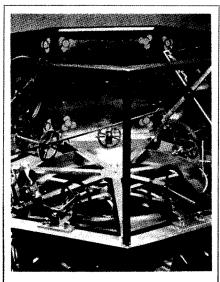
Characterisation of Solid Surfaces. Edited by P. F. Kane and G. R. Larrabee. Pp. xviii + 670. (Plenum: London and New York, 1974.) $39.00.

THESE two volumes bring out two immediate observations. First, that both the technology of producing surface coatings and the methods of microstructural analysis have undergone extremely rapid development. Second, that many of the new surface coating techniques still owe more to acute experimental observations and technical imagination than to direct application of scientific knowledge.

These thoughts are provoked particularly by the first volume on surface coatings, the editors of which deserve congratulations on producing in less than 500 pages 44 articles written by enthusiasts. These articles outline the bewilderingly large range of methods currently available for applying surface coatings to useful materials, and indicate some of their applications. It was clearly not possible or desirable in articles of this length to discuss any of the topics at all fully, but all of the articles give an outline of the physical principles involved and in most cases a useful list of references is included.

In addition to those topics that are predictably present—for example, electroplating, evaporation, chemical vapour deposition, and so on—there are other, more unexpected but worthwhile methods discussed. These include well established technologies such as brush painting, printing and spraying; the physics of such technologies being subjects less often considered in the scientific literature. Among the newer technologies, those of plasma arc and flame spraying and detonation bonding of refractory materials are described together with fascinating new ideas, such as that of 'electrical harmonic spraying' in which an electrical potential applied to liquid drops emerging from a tube can produce a collimated beam of liquid drops that are uniformly charged and have constant mass and velocity, such that they can be deflected accurately to form a deposited pattern without the need of a mask. Another idea that seems to be technically 'sweet' was the method of 'ion plating' in which adhesion between a substrate and a coating material can be improved by an increased kinetic energy of the first deposited layer. This increased energy is achieved by ionisation

of the evaporating atoms followed by electrical acceleration onto the substrate.

Two omissions, are, however, striking —'metalliding', the electrodeposition of metals with molten fluoride electrolyte was presented by abstract only; and no discussion was given of the Xerox process. The lack of a full discussion on metalliding was probably no fault of the editors, but at least a list of references might have been obtained from the missing author. The absence of Xerography is disappointing in that many of the coating methods discussed use charged material deposited onto a uniformly charged substrate, so selective deposition onto a partially charged

Modern reconstruction of Giovanni de Dondi's astronomical clock. The original was made in about 1364. From *Gears from the Greeks. The Antikythera Mechanism—A Calendar Computer From ca. 80 BC.* By Derek DeSolla Price. Pp. 70. (Neale Watson Academic: New York, 1975.) $8.50.

insulator seems a natural extension of these ideas.

The major criticism, however, must be that only a few articles discussed the *properties* of the coatings, and only one considered their structural assessment—either the title should have been 'Methods of Producing Surface Coatings', or more consideration of the properties of coatings should have been given.

Characterisation of Solid Surfaces is a very different volume in which a smaller range of topics (23) is considered in greater depth. The analytical methods described range from genuine atomic surface techniques such as Auger spectroscopy, electron spectroscopy for chemical analysis, and field ion microscopy, to techniques normally considered only for assessment of bulk microstructure such as transmission electron microscopy (TEM) and electron probe microanalysis (EPMA). The relevance of such bulk techniques is,

however, clearly justified in that the 'bulk' material studied—100 nm for TEM and 1 μm for EPMA—though large compared with a single atomic depth may be appropriate to many surface applications, such as surface coatings.

In addition, as shown by Laird in his excellent discusion of TEM, much genuine surface information can be gained by suitable specimen preparation facilities. The converse of this idea is also discussed—the analysis of bulk microstructure by use of surface analysis combined with erosion techniques so that the bulk can be revealed as a series of exposed surfaces. As a result of this interchange between bulk and surface analysis the content of this book will be of value to all interested in the microstructure of materials; not just to the rapidly growing band of surface scientists.

The techniques described all involve the illumination of surfaces with photons of various frequencies, electrons, ions or neutral atoms, followed by an examination of emitted photons, electrons, ions or atoms. The varied combinations of these techniques has given rise to a large industry both supplying and using increasingly expensive and varied equipment. It is noticeable that only in the chapter on optical microscopy was the cost of the equipment considered in respect to the value of the information obtained.

The rapidly growing arsenal of expensive equipment described indicates the importance of deciding what information is really needed in any application and which method is best for obtaining it. It often seems that scientists may become oriented to using just one technique—that in which they have detailed knowledge—ignoring others that may be more useful. The level of expertise obtainable from most of the chapters in this book should do much to indicate the advantage of other techniques, though for a detailed understanding one would have to make use of the literature cited.

Among the noticeable omissions was a discussion of low energy electron diffraction, though the editors refer to a good review. Perhaps more serious in view of the seemingly inevitable delays between writing and publication was the lack of a list of important references that might have covered the interval between writing and final printing of the volume.

A final and inevitable reflection on these two volumes is the lack of application of the analytical techniques to the evaluation of the structure of surface coatings; one can only hope that these two books will lead to some cross fertilisation between these two groups of surface scientists and technologists. **R. D. Doherty**

announcements

Awards

The Royal Geographical Society has announced the following awards: **Founders Medal** to **Sir Lawrence Kirwan** (geographical history of Nubian Nile Valley and Eastern Africa; services to exploration); **Patron's Medal** to **J. P. Kuettner** (exploration of Earth's atmosphere and oceans); **Victoria Medal** to **C. O. Sauer** (geographical research and education); **Busk Medal** to **N. N. Ambraseys** (historical seismicity and recent earthquakes in Near and Middle East); **Murchison Award** to **A. L. Mabogunje** (geography of West Africa); **Back Award** to **H. C. Brookfield** (geographical studies of Pacific region); **Peek Award** to **A. S. Goudie** (Late Pleistocene climatic phases in north-west India); **Gill Memorial** to **T. W. Freeman** (geography of Ireland); **Ness Award** to **A. Thompson** (botanical surveys in Guyana); **Cherry Kearton Medal and Award** to **Eric Ashby** (nature cinematography).

Appointment

T. G. C. Murrell has been appointed Foundation Professor of Community Medicine at the University of Adelaide.

Miscellaneous

Interstellar clouds. Professor A. Dalgarno, Professor of Astronomy at Harvard College Observatory, will give two Special University Lectures on Molecular Processes in Interstellar Clouds at University College, London (Gower Street, WC1) at 5 pm on April 23 and 25.

Geodynamics Project. The British National Committee for Geodynamics wishes to produce a comprehensive listing of papers relevant to this project, published by UK workers between January 1, 1971 and the present. Would such workers please send a bibliography of their papers to the Royal Society by April 30 (6, Carlton House Terrace, London SW1Y 5AG, UK).

Standard Telecommunications Laboratory. SRC and NERC are prepared to receive applications for the use of this facility at Harlow in Essex by university research groups for high pressure geophysical and geochemical experiments (Dr V. Essex, State House, High Holborn, London WC2, UK).

Person to Person

Oxford–Stanford. Oxford scientist requires three to four bedroom house in Stanford, California for sabbatical period, August 1, 1975–May 1, 1976. Possible exchange with modern four bedroom house in north Oxford, convenient for laboratories and all university buildings (Dr W. G. Richards, Physical Chemistry Laboratory, South Parks Road, Oxford, UK).

London flat. Canadian couple urgently require accommodation (flat rental) in London area from June, 1975–December, 1976. In return for assistance offered, willing to help with accommodation problems in Montreal, Toronto or Vancouver (Dr I. Altosaar, Department of Biochemistry, Imperial College, London SW7, UK).

London–Washington. Three bedrooms, central heating, available for exchange with accommodation in Washington, DC (within reasonable distance of National Cancer Institute). Approximate dates: August 1, 1975–July 1, 1976. Contact: M. Norman, 97 Highland Road, Bromley BR1 4AA, UK.

Boston–London. Two to three bedroom accommodation required for all or part of August in London or along the south coast, by a responsible academic family (two school children) from Boston area. Exchange arranged if interested. (Christine Armett-Kibel, 18 Devon Terrace, Newton Centre, Massachussetts 02159.)

Reports and publications

Great Britain

Bulletin of the British Museum (Natural History). Entomology. Vol. 31, No. 6: A Review of the Subfamily Oxyinae (Orthoptera: Acridoidea). By D. Hollis. Pp. 189–234. £2.75. Entomology, Supplement No. 24: A Revision of the Subfamily Coelidiinae (Homoptera: Cicadellidae), Tribes Tinobregmini, Sandersellini and Tharrini. By M. W. Nielson. Pp. 1–197. £12.20. Zoology. Vol. 27. No. 5: Catalogue of the Types of Terrestrial Isopods (Oniscoidea) in the Collections of the British Museum (Natural History). I. Superfamily Pseudotracheata. By R. J. Lincoln and J. P. Ellis. Pp. 189–246. £3.10. Vol. 26, No. 6: *Campylaspis* Species (Crustacea: Cimacea) from the Deep Atlantic. By N. S. Jones. Pp. 147–300. £3.25. (London: British Museum (Natural History), 1974 and 1975). [271

The Management of Broadleaved Woodlands. (Report of the Fourteenth Discussion Meeting, Institute of Foresters of Great Britain, Reading, 5 to 7 April, 1974.) (Supplement tp *Forestry.*) Pp. 119. (London: Oxford University Press, 1974.) £2. [271

Other countries

Slow Sand Filtration. By L. Huisman and W. E. Wood. Pp. 122. (Geneva: WHO; London: HMSO, 1974.) Sw.fr.16. [211

Agriculture Canada: Canadian Grain Commission. Grain Research Laboratory—1973 Report. Pp. vi + 92. (Ottawa: Information Canada, 1974.) [211

Environment Canada: Fisheries and Marine Service. Technical Report No. 490: Eggs, Larvae and Juveniles of Fishes from Plankton Collections in the Gulf of St. Lawrence During 1968. By A. C. Kohler, D. J. Faber and N. J. McFarlane. Pp. 105. (St. Andrews, NB: Research and Development Directorate, Biological Station, 1974.) [211

Académie Royale de Belgique. Memoirs de la Classe des Sciences. 2eSerie, T. XLI, Fascicule 5: Acritarches de la "Trancheé de Senzeille" (Frasnien Supérieur et Famennien Inférieur). Par F. Stockmans et Y. Willière. Pp. 79 + 4 planches. (Bruxelles: Académie Royale de Belgique, 1974.) [221

Smithsonian Contributions to Zoology. No. 175: On the Caobangiidae, a New Family of the Polychaeta, with a Redescription of *Caobangia billeti* Giard. By Meredith L. Jones. Pp. iii + 55 (11 plates). $1.15. No. 182: Spider Crabs (Crustacea: Brachyura: Majidae) from the International Indian Ocean Expedition, 1963–1964. By D. J. G. Griffin. Pp. iv + 35. 85 cents. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [221

United States Department of the Interior: Geological Survey. Professional Paper 832: Geology of the Skagway B–3 and B–4 Quadrangles, Southeastern Alaska. By E. M. MacKevett, Jr., E. C. Robertson, and G. R. Winkler. Pp. iv + 33. (Washington, DC: Government Printing Office, 1974.) $2.05. [231

Annual Report of the Institute of Earth and Planetary Physics for 1974. Pp. 117. (Edmonton, Alberta: Institute of Earth and Planetary Physics, University of Alberta, 1974.) [231

United States Department of the Interior: Geological Survey. Professional Paper 816: Geology of the Betterton Quadrangle, Kent County, Maryland, and a Discussion of the Regional Stratigraphy. By James P. Minard. Pp. iii + 27 + plate 1. (Washington, DC: US Government Printing Office, 1974.) $1.20. [231

A New Model for Level Areas. By Doeko Goosen. (Inaugural Address.) Pp. 32. (Enschede, The Netherlands: International Institute for Areal Survey and Earth Science, 1974.) [271

Australian Journal of Zoology. Supplementary Series. No. 31: The Generic Relationships of the Scincid Lizard Genus *Leiolopisma* and its Relatives. By Allen E. Greer, Jr. Pp. 67. No. 30: A Monograph of Australian Fleas (Siphonaptera). By G. M. Dunnet and D. K. Mardon. Pp. 273. No. 32: The Tipulidae (Diptera) of Australia. XII. The Genux *Dolichopeza* Curtis. By N. V. Dobrotworsky. Pp. 27. (East Melbourne, Victoria: Editor-in-Chief, Editorial and Publications Service, CSIRO, 1974.) [271

Bulletin of the Fisheries Research Board of Canada, No. 189: Treatment of Fish Processing Plant Wastewater. By F. G. Claggett and J. Wong. Pp. 18. (Ottawa: Information Canada, 1974.) $2.40. [271

Smithsonian Contributions to Zoology, No. 166: A Checklist of the North and Middle American Crayfishes (Decapoda: Astacidae and Cambaridae). By Horton H. Hobbs, Jr. Pp. iii + 161. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office). $2.50. [271

Records of the Dominion Museum, Wellington, New Zealand. Vol. 8, No. 12: New Species of Haustoriidae, Phoxocephalidae, and Oedicerotidae (Crustacea: Amphipoda) from Northern and Southern New Zealand. By R. D. Cooper and A. A. Fincham. Pp. 159–179. Vol. 8, No. 13: A New Subgenus of *Rhytida* Albera, 1860 (Mollusca: Pulmonata: Paryphantidae) from New Zealand. By F. M. Climo. Pp. 181–183. Vol. 8, No. 14: The Marine Algae of Stewart Island—a List of Species. By N. M. Adams, E. Conway and R. E. Norris. Pp. 185–245. Vol. 8, No. 15: New Species of Brittle-Stars from New Zealand (Echinodermata: Ophiuroidea). By Alan N. Baker. Pp. 247–266. Vol. 8, No. 16: Risso's Dolphin in New Zealand Waters and the Identity of "Pelorus Jack". By Alan N. Baker. Pp. 267–276. Dominion Museum Records in Ethnology No. 12: Cook Voyage 'Trading Stations' in Early Protohistoric New Zealand By D. Wayne Orchiston. Pp. 133–156. Report of the Board of Trustees, National Art Gallery, National Museum, and National War Memorial, for the year ended 31 March 1974. Pp. 27. (Wellington, NZ Dominion Museum, 1974.) [271

Radiological Factors Affecting Decision-Making in a Nuclear Attack. (NCRP Report No. 42.) Pp. viii + 66. (Washington, DC: National Council on Radiation Protection and Measurements, 7910 Woodmont Avenue, 1974.) [271

nature

nclude that there is no justification for our assertions, that more work is needed.

The work reported in this paper was supported by the Science Research Council who paid £15,000 for a project, the title of which can only be said to bear a remote resemblance to the title of this paper. This money paid for a highly sophisticated microscope which turned out to be unnecessary, a digitiser of a type which it later transpired was possessed by many other laboratories within walking distance, a chart recorder which has been useful on a couple of occasions, a trip to America to give a ten-minute lecture at 8.20 on the first day of a conference, numerous train journeys to London to attend committee meetings the value of which the entire committee agrees privately is nil and a research assistant whose use has been minimal but whom the professor could find nobody else able to pay for.

Capital facilities were provided by the University Grants Committee and include my large office and personal laboratory which unfortunately remained empty during my sabbatical year.

I thank my research students who, initially unable to see the point of my work, are now unable to see the point of anyone else's. I appreciate their good-humoured forbearance whilst I wrote their theses for them, persuaded the examiners that this was a significant contribution to knowledge and set them up in university careers of their own despite their initial belief that they could be more productive in industry.

I thank my secretary for patiently retyping this manuscript in its entirety several times when I have discovered grammatical and punctuation errors. Her cheerfulness in re-making forty photocopies of the manuscript and dispatching them each time by air mail to my friends and enemies is acknowledged. Her skill in finding things to do during my sabbatical absence is also appreciated. Admittedly the absence was mitigated by her being able to type my book, for which I shall receive a modest royalty, and being able to look after my other private business interests for which the facilities of the department are occasionally most helpful.

I thank my wife for acting as laboratory assistant. Her initial untutored clumsiness did not deter her from continuing to try hard. She was particularly helpful as my personal assistant on my trip to America.

The computational work described in the paper was done at the University Computer Centre whose staff I thank for their courtesy in helping me debug the program and in carrying over my paper output to me every day. They may be reassured that their work was not in vain— the final results agreed perfectly with predictions based on my pocket calculator. Although the amount of printing on each page of my output may have seemed to them somewhat slight, the paper is being put to good use for crayonning in my son's nursery school.

I thank the departmental tea-lady who never forgets to acquire, during her lunch break, a large cream bun which I particularly like to have delivered to my office with my cup of tea at four o'clock.

The equipment used in the experiments reported in this paper will not, as far as I can see, be used by me again. Nevertheless, I cannot, alas, see my way to letting anyone else use it, as it has certain characteristics which might confuse other users and there is always the possibility of damage. Likewise although the data which I have amassed is by no means completely described in this paper, I do intend some day to look once again at these results. In the meantime it would be inappropriate for anyone else to attempt to set up similar experiments; I am perfectly happy to share my unworked data with anyone. But I must point out that the information on the magnetic tapes is not self-evident, so anyone wishing to share would do well to visit my laboratory—I would happily co-author any paper that emerged from such a visit.

Finally, I must say something about this idea of accountability in science which some people are talking about these days. The thought that a researcher at the frontiers of knowledge should be spending the government's money and his own and his students' time other than in an efficient and conscientious way is quite alien to me and contrary to the spirit of academic freedom. I know I give value for money in my research and I need no small-minded bureaucrat telling me how to cut costs —besides 'value for money' is mercifully indefinable in the world of pure research. Yet, for what it's worth, I am prepared to say that I have rigorously observed the government's energy conservation measures. To conserve electricity, nobody on this project has been allowed to work in the laboratory during the hours of darkness. This has, of course, somewhat affected our productivity, but it reflects my concern that at a time like this we should not waste the nation's resources.

Metal-bending child: paranormal phenomenon? (Picture: Hemel Hempstead Gazette.)

In the past 18 months the scientific community has been confronted with renewed claims that certain people possess paranormal powers, including the ability to effect physical changes in materials. Work has proceeded in several scientific laboratories in an effort to come to terms with these apparent abilities. What sort of attitude should be adopted in attempting to verify these claims? First, J. B. Hasted, D. J. Bohm, E. W. Bastin and B. O'Regan describe their approach in recent work done at Birkbeck College, University of London. Then J. G. Taylor, of King's College, London, who is the author of a forthcoming book on his study of these phenomena, reviews the philosophy of his work.

IN February 1974 we made contact with Mr Uri Geller and were able throughout the year to observe psychokinetic phenomena at four sessions, in addition to participating in sessions with children. A brief report of our observations has been prepared for circulation to those interested. Among the claims made are that plasticity of metal was paranormally produced and that part of an enscapsulated single crystal of vanadium carbide apparently vanished. It is clear both to us and to the referees used by *Nature* that this account does not amount to a rigorous loop-hole-free report on a subject historically shot through with loop-holes. Nevertheless we believe that we have some significant work in progress, and the experience we have gained may be of value to other physicists interested, like ourselves, in the interactions between mind and physical systems.

We have come to realise that in this domain the experimental situation is different in certain crucial ways from that which has been common in scientific experimentation. This is because the phenomena under investigation have to be produced from the minds of one or more of those who participate. Relationships among the participants therefore play a much more essential role than is usual in traditional scientific fields. These relationships have to be taken into account in a way that is somewhat similar to that needed in the disciplines of psychology and medicine. But, of course, this does not mean that we are committed from the outset to the belief that paranormal phenomena are genuine. Rather our minds are open to all possibilities, and our wish is simply to discuss what is the truth about these phenomena. Indeed, our experiments contain features designed to clear up a number of doubts that naturally arise in this type of work. Nevertheless, in addition to being careful in this way, we have also to be sensitive and observant, and not to react with a preconceived pattern of tough-mindedness that will interfere with our perception, and that may destroy the very possibility of the phenomena that we wish to study. With proper sensitivity and perceptivity we attempt to develop an approach that will adequately allow for the interpersonal element and yet permit us to engage in valid scientific research.

One of the first things that reveals itself as one observes is that psychokinetic phenomena cannot in general be produced unless all who participate are in a relaxed state. A state of tension, fear, hostility, on the part of any of those present generally communicates itself to the whole group. The entire process goes most easily when all those present actively want things to work well. In addition, matters seem to be greatly facilitated when the experimental arrangement is aesthetically or imaginatively appealing to the person with apparent psychokinetic powers.

We have found also that it is generally difficult to produce a predetermined set of phenomena. Although this may sometimes be done, what happens is often surprising and unexpected. We have observed that the attempt to concentrate strongly in order to obtain a desired result (the bending of a piece of metal, for example) tends to interfere with the relaxed state of mind needed to produce such phenomena. It appears that what is actually done is mainly a function of the unconscious mind, and that once the intention to do something has been firmly established, the conscious functions of the mind, in so far as they have bearing on the goal, tend to become more of a hindrance than a help. Indeed, we have sometimes found it useful at this stage to talk of or think about something not closely related to what is happening, so as to decrease the tendency to excessive conscious concentration on the intended aim of the experiment. A comparison might be made with the process of trying to go to sleep, for which is needed a firm intention, without subsequent efforts.

Many of the conditions described above are also required for fruitful research in the natural sciences. Thus, if any of those who participate in a physical experiment are tense and hostile, and do not really want the experiment to work, the chances of success are greatly diminished. Likewise, the aesthetic appeal of the experimental set-up often helps to maintain interest and enthusiasm, whereas an attitude that consistently tends to damp these latter is evidently detrimental to the whole enterprise. In the study of psychokinetic phenomena, such conditions are clearly much more important than in the natural sciences, because the person who produces these phenomena is not an instrument or a machine. Any attempt to treat him as such will almost certainly lead to failure. Rather, as indicated earlier, he must be considered to be one of the group, actively cooperating in the experiment, and not a 'subject' whose behaviour is to be observed 'from the outside' in as cold and impersonal manner as possible.

The following analogy may help to give a more orderly overall description of the phenomena in the field. Consider a person whose hand has been paralysed as a result of destruction of nervous tissue. If this person is to regain the use of his hand, he must somehow

activate new nervous pathways. How he is to do this, he does not know. All he can do, with all his energy, is to feel out the possibilities of movement and to observe with great attention and alertness what movements actually take place. He cannot describe or even think about just what it is that he does in getting his hand to move. Moreover, he cannot at first produce controlled movements, which bring about consciously intended results. Rather it is clear that the contact between brain and hand is brought about almost entirely by unconscious functions of the mind, which tend to be erratic and fortuitous. Of course, if he works with sustained interest and energy, he will generally find that his movements do begin to come closer to what he intends. But it is also clear that if he is surrounded by people who are not open to the possibility that he can move his hand or who bring about a state of psychological tension through hostility, then he will be less likely to be able to sustain the interest and energy needed for learning how to move his hand.

Those who work with such a person (for example, the physiotherapist) must evidently not be committed to resisting the notion that the hand can eventually move. For the thoughts in the paralysed person's mind, and those in the minds of his colleagues, are both important factors in bringing about success. The necessary open-ness to the possibility of an ultimate result must be maintained in the minds of all concerned, while at the same time there is a healthy capacity to be tentative and free of definitive conclusions in statements about what particular results may have been achieved at a certain stage. And so reliable inferences can be made in physiotherapy, though by methods that are rather different from those used traditionally in the natural sciences.

The analogy between this field and that of psychokinetic research is fairly clear. The main difference is this: we can account for and to some extent explain the connection between the brain and the hand in the case of the paralysed person (through the nerves which link them) but we have no way either to account for or to explain the connection between the brain and the object that is moved, bent and so on, in terms of what is now known to science. If, however, we suppose that there is some at present unknown force, energy or mode of connection, then we may also suppose that psychokinetic power may function in a way that is essentially similar to the power to move the hand. Thus, one might suggest that perhaps there is an unconscious 'feeling out' of the connection. In many cases there is a visible 'feedback' that enables a person to

recognise that he has done something, and that permits him to try to go further along the same (indescribable and undefinable) lines. But there may other forms of 'feedback'. Thus, if a piece of metal can respond to the brain in an unknown way, the brain may similarly respond to the metal. By being sensitively aware of this response, the person concerned might be able to tell when something had actually happened, even though the object in question was not sensually perceptible to him.

It is important, at this point, not to insist on having a potential theoretical explanation before one will seriously consider observing the phenomena themselves. Thus, when magnetic and electrostatic effects were first observed, it was impossible to account for them in terms of the known forces, which were considered to arise only when bodies were in mechanical contact. Evidently, this did not prevent the effects from being observed. The main aim of such observation is to give rise to an orderly account of the phenomena, which is first qualitative and

then quantitative—for example, first the qualitative observation that like charges repel, unlike charges attract, and then the quantitative observation that the force is inversely proportional to the square of the distance. On the basis of such an account, current field-theoretical explanations of electromagnetic phenomena were later developed. We propose a similar approach to psychokinetic phenomena, and in our work thus far we have tried to carry it out.

In such research an attitude of mutual trust and confidence is needed; we should not treat the person with psychokinetic powers as an 'object' to be observed with suspicion. Rather, as indicated earlier, we have to look on him as one who is working with us. Consider how difficult it would be to do a physical experiment, if each person were constantly watching his col-

leagues to be sure that they did not trick him. How, then, are we to avoid the possibility of being tricked? It should be possible to design experimental arrangements which are beyond any reasonable possibility of trickery, and which magicians will generally acknowledge to be so. In the first stages of our work we did in fact present Mr Geller with several such arrangements, but these proved to be aesthetically unappealing to him. From our early failures, we learned that Mr Geller worked best when presented with many possible objects, all together on a metal surface; at least one of these objects might appeal to him sufficiently to stimulate his energies. In a later session, we had such a set-up, which included two small plastic capsules, each containing a thin disk of vanadium carbide single crystal. A clearly observable change in the disk within one of the capsules was brought about when Mr Geller held his hands near them.

In discussions with magicians we have learned that the best conditions for a conjuring trick arise when the happening significantly precedes the observation. In the above instance we believe that the conditions were such that the failure to observe and record the precise moment of change is of no importance, because there is no known way of producing this effect within the closed capsule and no possibility of substitution. For this reason we conclude that this was something that no magician could have done.

Nevertheless, we realise that conditions such as we have described here are just those in which a conjuring trick may easily be carried out. We understand also that we are not conjuring experts, so that if there should be an intention to deceive, we may be as readily fooled as any person. Moreover, there has been a great deal of public criticism, in which the possibility of such tricks has been strongly suggested. For this reason it has often been proposed that a skilled magician should be present, to help to see to it that there will be no possibility of deception.

It is in the nature of the case, however, that no such assurance can actually be given. For a skilled magician is able to exploit each new situation as it arises in a different and generally unpredictable way. The corpus of tricks is not fixed, but rather continually changes and evolves. A particular magician could therefore say at most that he knew of no tricks that could have brought about a given set of observed phenomena. Of course, if several magicians of recognised proficiency were to conclude that what was done on a certain occasion did not in-

volve any tricks, this could help create a presumption in favour of the notion that the phenomena are genuine. In principle, we would welcome help of this kind in decreasing the possibility of deception. It has been our observation, however, that magicians are often hostile to the whole purpose of this sort of investigation, so that they tend to bring about an atmosphere of tension in which little or nothing can be done. Indeed, even if some magicians were found who were not disposed in this way, it does not follow that their testimony will convince those who are hostile, since the latter can always suppose that new tricks were involved, beyond the capacity of those particular magicians to see through them. Because of all this, it seems unlikely that significant progress toward clearing up this particular question could be made by actually having magicians present at the sessions, though we have found it useful to have their help in a consultative capacity. We have learned in such consultations not to withdraw our scrutiny from the identified specimen, from the first moment when it reaches the hand of the subject until the bend occurs. We are familiar with the use of the human hair in producing small movements, with the use of mercuric salts in alcohol to corrode metals, and with the weakening in metals produced by continued bending to and fro. We recognise that there is a genuine difficulty in obtaining an adequate answer to criticisms concerning the possibility of tricks, and that a certain healthy scepticism or doubt on the part of the reader may be appropriate at this point. Indeed, it would be inappropriate if the scientific community did not at first react in such a way. We believe, however, that our approach can adequately meet this situation.

It is essential that in at least some experiments, conditions must be controlled in such a way that the possibility of deception is insignificant. Metal bending and cleavage experiments are particularly suitable for this approach. Encapsulated specimens can play an important part, although up to the present we have only been able to achieve success with one such specimen.

We feel that if similar sessions continue to be held, instances of this kind might accumulate, so that there will be no room for reasonable doubt that some new process is involved here, which cannot be accounted for or explained in terms of the laws of physics at present known. Indeed, we already feel that we have made sufficient progress toward this point to warrant further investigations along these lines. We hope to carry out more tests and to report on them when results are available. ☐

It is not easy to discern a sharp boundary at which scientists must stop and turn into magicians . . .

THE crucial question that any scientist has to ask himself if he wishes seriously to investigate the paranormal (spoon-bending, telepathy, clairvoyance and the like) is how to do so scientifically. There are those who claim the very elusiveness which paranormal events have in their nature precludes any such approach. But there are also persons like myself who feel that these strange phenomena are relevant to their world view and so deserve careful study. Do we necessarily have to doff the garb of scientist when satisfying our curiosity about such events?

We can say at the outset of attempting to answer this question that if the response were "yes", science would be circumscribed in a very peculiar way. No elusive phenomenon would be allowed as a suitable object of scientific enquiry; on this score quarks, for example, would be beyond the scientific pale. To avoid such absurdity we must realise that there is a continuum of levels of elusiveness. It is not easy to discern a sharp boundary at which scientists must stop and turn into magicians.

There is one feature, however, which should be present in any phenomenon to be investigated scientifically. That is repeatability, allowing the possibility of many investigators observing the event at different times. Not only does this allow the 'consensibility' to be practised which is so crucial to scientific investigation but above all it permits the phenomenon concerned to be probed ever more deeply. Only then can the event begin to be seen in terms of current understanding. Thus the two processes of validation and model building can be carried out, possibly hand in hand or almost simultaneously.

At this point the notion of repeatability for paranormal phenomena must be clarified, since it seems to mean different things to different people. It is being used here in the following sense. Suppose that an event, such as a child causing a spoon to bend, has occurred a number of times in a given environment. The bending may not happen every time the child is tested, possibly because some crucial factor in the surroundings has changed from one test to the next without anyone realising. Yet provided that in a non-zero fraction of the test situations spoon bending (or whatever it is) has occurred and enough tests have been made to feel confident that the phenomenon will continue at about the same rate then the para-

normal event will be termed repeatable.

One of the main difficulties in applying this criterion is in the specification of the precise environment in which the event will recur. There can well be psychological pressures put on the subject by the presence of particular objects or persons, and the most suitable environment has to be found by laborious trial and error. But these problems need not make one despair of obtaining repeatability. In particular subjects should not be expected to cause a paranormal event every time they are asked to, and in any surroundings. It is like expecting a particle accelerator to produce its beam of particles whatever level of vacuum there is in the accelerating tube or whatever potential is applied. The design of such a machine is so precise that it can only function under very special conditions. We are in the position of dealing with even more complicated machinery in human beings and account must be taken of that when investigating the paranormal.

In order to be able to investigate a phenomenon further, it is necessary that it can occur under suitably modified conditions. If spoon bending were only ever possible when the subject could not be directly observed during the bending process then, even if it were repeatable, a number of explanations for it, including the obvious one, would be possible. In general it is clear that conditions must be able to be chosen so that simple explanations of paranormal phenomena, especially trickery, are ruled out.

We come, then, to the clash between the eventual rigour of science and the laxity apparently needed to produce paranormal phenomena. Seances need to be held in the dark since the light of day seems to prevent anything strange occurring. This is not true of spoon bending or distant viewing, for which there is some level of repeatability in daylight. There are other phenomena, such as dowsing, which can also bear further investigation of a sort which allows various explanations to be tested.

It is this feature of allowing critical testing which is a *sine qua non* of a phenomenon for its investigation to be regarded as scientific. The range of tests used will naturally be at the discretion of the investigator, but it is in the event itself that this 'testability' resides. Not that further enquiry need prove easy, since it will involve modifying the environment in some way that may prevent the phenomenon from recurring. If any such change is too inimical to the event then clearly it is not possible to test it at all; the event will have to be regarded as a curiosity, but one which at present cannot usefully be studied scientifically.

As new tests are tried with a subject,

the phenomenon may initially disappear but then return at a later stage after several attempts. The investigator clearly requires patience in such a situation, but this must be exercised since otherwise the phenomenon remains too poorly investigated. It may be necessary to do this gradually, but somehow or other the phenomenon must be delimited precisely enough that it can be validated and that conjecture as to its possible explanation can begin. In particular, tests must be introduced which show with utmost rigour that fraud can be excluded as the cause of the events. Provided these tests are conclusive, then the phenomenon can be probed further.

An example of this is spoon bending, which has been observed by many investigators during the past two years. Some of the accounts are valuable, though not conclusive by the above criteria. Only by careful measurement of various physical parameters during the bending process, especially the pressure applied, can the phenomenon be classified as paranormal. The measurement process itself would best be done automatically, as has been arranged in some preliminary tests with positive results. Measurement of various parameters such as temperature and radiation levels of various sorts have also been attempted. In all of these attempts care has had to be taken to relax the subject as much as possible, yet always to avoid simultaneously relaxing scientific rigour.

The problem of preventing stress by the presence of the sceptical observer seems to be handled best by using as much automatic measuring apparatus as is feasible. The investigator can then devote more of his attention to the psychology of the subject than would be the case if he had to divide his time between trying to keep the subject relaxed and observing precisely what was occurring. Film or video tape have not proved as useful here as one would like, since they only provide a single view of the event. And they cannot monitor crucial variables such as pressure or magnetic field strength.

Finally we come to the sceptic in whose presence the phenomena may not occur or who has not been able to observe the events and considers them too strange ever to be possible. Such a critical attitude plays an important role in science, though over-reaction can take place if the sceptic is too emotionally involved in the event to preserve his objectivity. Even this serves the purpose of ensuring that standards of scientific rigour are preserved. But advance in the paranormal field will be held up by too sceptical an attitude. The question to be settled is what sort of evidence would be accepted by the sceptic? The answer to this clearly

depends on the particular sceptic involved, but certain general features of such an attitude can be discerned.

The most extreme sceptic is one who will never budge from his sceptical attitude, whatever is presented to him about the paranormal. Such an obdurate attitude can only be attacked by senility and death, so we turn to the slightly less sceptical. He requires to observe the phenomenon at first hand, since it seems for him such an impossible occurrence. Yet, even when he has observed it, he may claim that fraud had occurred. He must be asked to observe in situations with all possible rigour applied. Time and patience will be needed by him to obtain results; only if he can be influenced to expend them may he be able to observe under conditions which will convince him.

It has been assumed here that it would be possible to obtain conditions for observing paranormal events under which fraud is impossible. Some claim that this is not so, but this is clearly false. It is always possible to choose conditions in which trickery can be completely excluded. A piece of metal to which a pressure sensor is attached cannot be bent surreptitiously unless the sensor is inoperative. If the method of bending were non-mechanical, such as by the application of chemicals or by the use of a laser beam, careful chemical analysis or temperature measurements would indicate this. An object caused to be distorted without contact cannot have become so by normal means.

It is not possible at present for all such sceptics to be able to observe careful tests performed on the too few subjects with apparent paranormal powers. Nor does it seem that even a fraction of them will ever be able to do so. To counter such a weight of scepticism, it is necessary to consider its source in more detail. At the root of scientific scepticism of the paranormal is the fact that the events being investigated are in contradiction with present scientific understanding. At least, it is claimed that this is a fact for all paranormal events, but there are differences between different sorts of phenomena.

Firstly there are strange events which seem to be scientifically impossible, on the face of it, but which might possibly have a scientific explanation in the future provided that the present lack of knowledge in the relevant area is acknowledged. A case in point is, again, spoon bending, for which an electromagnetic explanation has recently been suggested. To test this properly requires considerable research into the interaction of especially low frequency electromagnetic radiation with various materials up to the point of their fracture. Only very recently has a new

feature of the latter been discovered in support of the hypothesis, in that transient electric and magnetic fields have been measured in association with tensile fracture of various metals. A similar hypothesis can be put forward for telepathy and distant viewing. Recent results from the group at the Stanford Research Institute (Targ and Puthoff) indicate an upper limit to bit-rate transfer which would again implicate very low frequency radiation. Since low frequency electromagnetic radiation has been shown to have effect on psychological parameters of animals, such an electromagnetic hypothesis might just be considered.

Other physical phenomena are more difficult to understand in such terms. Thus, if objects can truly be caused to disappear from closed containers then the energy required to dissolve chemical bonds is so enormous that no feasible physical explanation can be given in present terms. The phenomena of this type are not as well attested as those of spoon bending and distant viewing, so there is still a possibility that 'travelling' objects are not totally disappearing in one place and reappearing in another. If they are, however, then clearly a contradiction has occurred with current scientific understanding of the forces of nature and the principles they satisfy, especially that of conservation of energy.

Similar difficulties arise with other paranormal events such as precognition. That is also hard to put into the usual causal framework, which uses retarded potentials as solutions of the field equations for all the forces. The advanced potential solutions have been attempted in the past, though it would be strange if such advanced effects only arose when human beings were present. Naturally enough it is also necessary to consider the part which consciousness plays in these phenomena. A consistent picture of the mind as constructed from material building blocks is far more feasible than a tachyon-like picture which at present has currency (especially because of the complete lack of evidence for the existence of tachyons).

In conclusion, it would seem that paranormal events can be investigated scientifically provided the investigator uses his patience and ingenuity to the full, both with his subjects and with his colleagues. He must aim for the highest level of scientific rigour in his tests, though be prepared to spend a great deal of time before a subject can cause a paranormal event to occur in scientifically satisfactory conditions. Furthermore the investigator must attempt to make feasible models of the events. If all goes well, he will make enough progress to build his own machine to achieve the effect. □

international news

WHEN Congress put a stop to development of an American supersonic airliner five years ago, looming large in its decision was a controversial theory put forward by Harold Johnston of the University of California, Berkeley. Johnston argued that the aircraft would emit pollutants into the stratosphere which would partially destroy the ozone layer, the ultimate consequence of which would be a large increase in the amount of harsh ultraviolet radiation reaching the Earth's surface and a corresponding increase in the incidence of skin cancer in the Northern Hemisphere. The theory has been under strong attack ever since it was first put forward, but last week Johnston was vindicated by an unusually blunt report published by a committee of the National Academy of Sciences.

The report stated that operation of 300 to 400 of the Boeing supersonic aircraft then under consideration would "in all probability" have caused a 20% increase in skin cancer in the United States—that translates to about 80,000 extra cases a year. To emphasise the point, the report added that "a few additional people would probably have died each year and a few hundred people would probably have contracted skin cancer each year, for each formerly

NAS report spells trouble for Concorde

by Colin Norman, Washington

projected US SST put into service".

Those conclusions are of more than academic interest, for they almost certainly spell more political trouble for the financially beleaguered Concorde supersonic aircraft in the United States. The immediate effect of the report is likely to be to strengthen moves to deny Concorde landing rights at American airports.

The committee's chief conclusion is that a fleet of 100 Concordes or Soviet TU-144s, operating in the stratosphere for about 5 hours a day, would cause a reduction of 0.7% in the ozone layer. The resulting increase in ultraviolet radiation reaching the Earth would be about 1.4%, and that would be expected to lead to an increase in the incidence of skin cancer in the United States of about 1.4%, or 6,000 extra cases a year.

Concorde is expected to pose less of

a hazard than the scrapped Boeing SST because it will fly at lower altitudes and emit smaller amounts of the oxides of nitrogen—the chief pollutant responsible for depleting the ozone layer. But the problem is not limited solely to supersonic aircraft, the committee notes, because wide-bodied jets also fly in the lower stratosphere and inject oxides of nitrogen into it. If the subsonic fleet grows in size, and if the aircraft are designed to fly higher, as has been predicted, then it would pose just as serious a threat to health as a large fleet of Concordes.

Those effects can at least be mitigated by some costly engine modifications, however, and the committee therefore suggests that international regulations should be drafted to ensure that cleaner engines are used on aircraft flying in the stratosphere in future years. As a rough estimate, committee members suggested at a press conference last week that it would cost about $100 million for research and development on engine redesign, and that it would take between 10 and 15 years to equip aircraft with the cleaner engines.

The committee's conclusions are essentially the same as those reached by a three-year study conducted by the Department of Transportation which

SUPERSONIC airliners are not the only potential violators of the ozone layer. In fact, in view of the dismal economic outlook for Concorde and the enormous costs of operating the aircraft, the SST fleet is unlikely to become large enough to represent much of a threat to human health for many years, in which case there is probably sufficient time to develop cleaner engines to guard against potential hazards. Of more immediate concern is the possibility that propellants used in aerosol spray cans—chlorofluoromethanes, which are more commonly referred to by their trade name, Freons—may already be depleting the ozone layer at an alarming rate, and there has recently been a flurry of activity in the United States to try to assess the dimensions of that hazard.

In February, the federal government announced that it has established a top-level scientific committee to examine the potential dangers from aerosol sprays, and that it hopes to have a report by mid-June. Last month, the National Academy of

Sciences also announced that it has established a committee to look into the problem, and named Dr H. S. Gutowsky, Director of the School of Chemical Sciences, University of Illinois at Urbana, as its chairman. The study is expected to be completed within a year. The academy decided to establish the committee after an *ad hoc* panel, which met under the chairmanship of Donald M. Hunten of Kitt Peak Observatory, had suggested that there is an urgent need to determine the possible danger from aerosol propellants. After that panel had made its recommendations, Hunten suggested at a press conference that, in his opinion, the injection of Freons into the atmosphere should be halted as rapidly as possible and that a moratorium on their use should be maintained until the potential dangers have been assessed.

According to some estimates, Freons may already have been responsible for depleting the ozone layer by about 1% and, if their use continues unchecked, the depletion may

increase to about 5% by the 1990s.

Another concern that has recently been expressed is that extensive operation of the space shuttle will pose a threat to the ozone layer because it will inject hydrogen chloride into the stratosphere. NASA is now studying the basis for that concern.

Finally, NASA announced last week that it will use the Copernicus Orbiting Astronomical Observatory to monitor the amount of chlorine in the stratosphere. Freons are thought to pose a threat to the ozone layer because ultraviolet radiation may break them down in the upper atmosphere, to release chlorine, which then breaks down ozone molecules. Thus, by measuring chlorine in the stratosphere over long periods of time, it should be possible to determine the extent of the threat to the ozone layer. This will be done by comparing the ultraviolet radiation from a bright star which is absorbed at the wavelength characteristic of chlorine, first when the star is high in the sky and again when it is on the horizon.

was published earlier this year. Called the Climatic Impact Assessment Project (CIAP), that study resulted in an estimate that a fleet of 100 Concordes flying in the stratosphere for 4.4 hours a day would deplete the ozone layer by about 0.4%. The two estimates are well within the uncertainty range of the calculations, and Dr Henry M. Booker, the chairman of the academy committee, suggested in a statement last week that both of them indicate that "strict measures might be needed to protect the environment from the effects of future large-scale flight operations in the stratosphere".

Yet another report on the environmental effects of supersonic aircraft is expected to be published soon by the British Meteorological Office's Committee on the Meteorological Effects of Stratospheric Aircraft (COMESA). According to pre-publication snippets provided recently by Dr R. J. Murgatroyd, COMESA's chairman, the report will conclude that 1,000 Concordes would be expected to reduce the ozone layer by about 3%.

Much has been made of the fact that all those studies predict that the effects of Concorde on the ozone layer will be much smaller than natural fluctuations—concentration of stratospheric ozone can vary by as much as 25% from day to day, for example. But the academy committee notes that aircraft flying in the stratosphere will cause a systematic reduction in ozone concentration, superimposed on the daily fluctuations.

"Remember that skin cancer is an integrated effect (of exposure to ultraviolet radiation) over a long period of time", Booker said last week, and he noted that average exposure to ultraviolet radiation seems to be the chief factor which causes the incidence of skin cancer to vary with latitude.

Because the ozone layer is much thinner at the equator than it is at the North Pole, the amount of ultraviolet radiation reaching the Earth's surface at low latitudes is much greater than at high latitudes; sure enough, geographic variations in the incidence of skin cancer are closely correlated with geographic variations in ultraviolet flux.

The upshot of those conclusions is that if either the supersonic fleet or the fleet of high-flying subsonic aircraft is increased in size, or if a new generation of supersonic airliners, designed to fly at greater altitudes, is brought into service, potentially serious health hazards may result. The academy committee therefore suggests that regulations should be drafted post-haste to ensure that cleaner engines are developed and installed. It suggests that the best forum for drafting such regulations is the International Civil Aviation Organisation, and the World Meteoro-

IN exceptionally blunt language, a high level advisory committee last month expressed concern about the ordering of priorities within the much-publicised cancer research programme in the United States. In particular, the committee indicated its "astonishment" at the low priority accorded to studies on environmental carcinogenesis, and it took a swipe at the massive amounts of money that have been poured into research on possible human cancer viruses.

The committee, a special subcommittee of the National Cancer Advisory Board, said in a report delivered to the board last month that although there is "widespread recognition of the importance of environmental c h e m i c a l carcinogenesis", the National Cancer Institute (NCI) seems to have given such studies a low priority in terms of expenditures. Less than 10% of the cancer budget is spent on research into the environmental causes of cancer, the committee estimates.

Those concerns are the latest in a long line of grumbles from biomedical scientists about the content and direction of the so-called war on cancer. The grumbles have mostly been concerned with the fact that the cancer programme has been soaking up funds at the expense of other areas of biomedical research, and that it has adopted a highly targeted approach which has emphasised applied rather than basic research. But in this case, the committee is arguing that an area of research dealing directly with cancer control and prevention is being relatively neglected.

The report, which resulted from two days of meetings by the subcommittee under the chairmanship of Philippe Shubik, Director of the Eppley Institute for Research in Cancer, University of Nebraska, lists a number of recommendations for increasing the research effort on environmental carcinogens. The preamble to the report describes the committee's concerns in graphic language.

"There was an obvious sense of general astonishment throughout the meetings that the National Cancer Programme does not appear to have accorded an adequate priority nor sense of urgency to the field of environmental carcinogenesis, particularly when this concerns chemical carcinogens", the committee states. Later in the report, the committee suggests that "epidemiology thus far seems to suggest that a viral etiology for most human cancers is an unlikely eventuality; in view of this, the distribution of the budget of the NCI in the area of etiology with its emphasis on viral oncology should perhaps be reconsidered; perhaps the time is ripe for a reordering of priorities or at least for an in depth examination of this basic conclusion".

But the committee recommends that "the NCI should foster the development and validation of new and innovative bioassay techniques" for detecting and identifying potential carcinogens in the environment. In particular, the committee suggests that more special centres for research on environmental carcinogenesis should be established in the United States.

logical Organisation.

Although no threat is likely to be posed to the ozone layer by the handful of Concordes now scheduled to enter service, the academy's report is likely to strengthen opposition to any operation of the aircraft in the United States. It should be noted that the report has been published at a crucial moment, for public hearings open on April 14 on a request by British Airways and Air France for permission to land Concorde at John F. Kennedy Airport in New York and Dulles International Airport in Virginia, for a small number of proving flights later this year.

Last month, the Federal Aviation Administration (FAA) tentatively recommended that such permission should be granted, but a final decision will not be made for several weeks. In an environmental impact statement on the request for landing rights, the FAA noted that Concorde's engines emit substantially greater levels of some pollutants—particularly carbon monoxide—

and create more noise than any other passenger aircraft, but it suggested that "the environmental consequence of the limited volume of Concorde operations requested are not so severe as to compel a refusal (of the request)". Opponents, of course, did not agree.

Meanwhile, a bill has been introduced into the House of Representatives by Lester Wolff, a New York Congressman, which would prevent Concorde from landing anywhere in the United States unless it meets the federal noise and pollution standards which apply to subsonic aircraft. A few days before the FAA announced its tentative recommendation to grant the request for landing rights for Concorde, the Environmental Protection Agency proposed noise standards for supersonic aircraft which would be less stringent than those for subsonic aircraft. Hearings will be held on Wolff's bill in July by the Public Works Committee and, if passed, it would effectively deny Concorde access to the United States. □

Seminar contacts under pressure

from Vera Rich, London

THE increasing pressure on the remaining members of the Voronel Sunday seminars now seems to be spreading to their contacts throughout the Soviet Union. In Tbilisi, pressure is being exerted on the Goldshtein brothers, Isai (35) and Grigorii (42), both cyberneticists and both "refusniks" since 1971, when their applications to emigrate to Israel were rejected on the grounds of "security".

After they were refused their visas, the brothers were both dismissed from their posts at the Institute of Cybernetics of the Georgian Academy of Sciences. It is reported that the director of the institute admitted in a private conversation after their dismissal that neither brother had ever been connected with secret work but that he, the director, was "afraid".

The Goldshtein brothers have maintained close contacts with the members of the Moscow seminar, especially with Aleksandr Lunts and Viktor Brailovskii, who, since Voronel's departure for Israel, have been the leading organisers. The Goldshteins had planned to visit Lunts and Brailovskii at Passover, but were prevented from doing so by the authorities. After this attempt, they were taken to their local KGB offices, questioned about their activities and warned that their files would be handed over on April 7 to the State Prosecutor in preparation for a trial. It was also intimated that the

The Goldshteins: Isai (left). Elizaveta Bykova and Grigorii

charges against them would be linked with four other trials now being prepared in the USSR. Since Lunts and Brailovskii have already been threatened with legal proceedings, it is feared that they will be involved in these other trials.

The charge against the Goldshtein brothers falls within the category of an unpublished edict of the Presidium of the Supreme Soviet of 1972. It is alleged that since they were refused permission to go to Israel, they have been slandering the USSR, and that their activities threaten the security of the USSR—a charge which carries a maximum sentence of seven years in a strict regime labour camp.

So far, no charge has been preferred

against Elizaveta Bykova Goldshtein, the wife of Isai and, like him, a physicist. Since, however, she is the author of a survey of the problems and backgrounds of refusniks and the means used to intimidate them, which has recently reached the West through *samizdat* channels, it seems not unlikely that she too could share the fate of her husband and brother-in-law.

In the week that physicist Evgenii Levich, who was associated with the Voronel seminar, was finally allowed to emigrate to Israel together with his brother, Aleksandr (an engineer), it seems ironic that two other brothers, also associated with the seminar group, should attract the renewed attentions of the KGB. ☐

"A major climatic change would force economic and social adjustments on a worldwide scale . . . it is not primarily the advance of a major ice sheet over our farms and cities that we must fear . . . rather, it is persistent changes of the temperature and rainfall in areas committed to agricultural use, changes in the frost content of Canadian and Siberian soils, and changes of ocean temperature in areas of high nutrient production . . . Our vulnerability to climatic change is seen to be all the more serious when we recognise that our present climate is in fact highly abnormal".

These sentiments will already be familiar to readers of *Nature*; now they come with the official blessing of the US National Academy of Sciences in a report, "Understanding Climatic Change—A program for action" prepared as part of the US contribution to the Global Atmospheric Research Program (GARP). There is little in the report that will be new to the serious student of climatic change, although a lengthy appendix surveying past

climates does pull together in one handy review a wealth of scattered information; but the Panel on Climatic Variation which has produced the report makes some far-reaching recommendations about the nature of future research:

● The immediate adoption and development of a coherent National Climatic Research Program to include

● A Climatic Data Analysis Program with the development of new climatic data-analysis facilities and

● A Climatic Index Monitoring Program to acquire the needed data with

● A Climatic Modelling and Applications Program to accelerate research on climatic variation.

The Panel sees these American efforts as best taking place within an international framework, and suggests that the period prior to 1980 "be used to develop additional scientific and technical manpower through the establishment and support of fellowships in appropriate areas of climatic research", and that the period 1980–2000 should be designated the "International

Climatic Decades" during which data should be gathered extensively on an internationally collaborative basis and regional climatic studies of anomalies of special interest carried out.

Apart from the rather leisurely time-scale mapped out (in view of the present world food situation and the changes in climate that have occurred in the past 25 years) the report is likely to be welcomed by all of those climatologists whose lonely warnings about the importance of climatic change are now provided with the seal of scientific respectability. The cost of the massive effort needed (massive by scientific standards, but small compared with, say, the development of a nuclear missile system) must make it doubtful whether a really effective programme will take place. That is in the hands of the politicians; but politicians outside the USA should also take note of this NAS report. They may decline to take appropriate action, but they cannot say any longer that they do not have the necessary information on which to base a course of action. *John Gribbin*

correspondence

The NPT and the IAEA

SIR,—Your editorial on "The unloved treaty" (March 13, 1975) shows how important it is to exercise the utmost care in reaching conclusions about political as well as scientific matters The gist of the article is that the foundations of the Non-Proliferation Treaty (NPT) "show signs of crumbling" and that it has failed "to catch on"

It is strange to read this at a time when all the non-nuclear-weapon countries of the EEC are at the point of completing the process of ratification of the treaty, when the Government of Japan is reported to be putting the treaty to the Diet in April for discussion and ratification and indeed when many countries are seriously concerned to prevent further nuclear proliferation and to increase the effectiveness of IAEA safeguards During the past year we have witnessed several steps taken by the main supplier countries in this direction, such as an agreement on a list of nuclear equipment and materials which will "trigger" the application of safeguards requirements for safeguards on specialised technological information and an increased interest in safeguards in countries that have previously been somewhat indifferent

The editorial's comments on the peaceful uses of nuclear explosions are, I believe, equally inaccurate Article V of the NPT specifies that the potential benefits of peaceful applications of nuclear explosions be made available to non-nuclear-weapon states party to the treaty "under appropriate international observation and through appropriate international procedures " And further, "n o n - nuclear-weapon states party to the treaty shall be able to obtain such benefits, pursuant to a special international agreement or agreements, through an appropriate international body with adequate representation of non-nuclear-w e a p o n states"

When you ask "so where is the international body?" you overlook that the IAEA, with 106 member states, would clearly seem to meet the requirements set out in NPT and that there is no need to establish a new international organisation In 1968, the United Nations Conference of non-nuclear-weapon states recommended that the agency initiates studies on its possible functions in the field of peaceful nuclear explosions (PNE), and the agency's suitability in this respect has

been specifically recognised by its own General Conference and by the United Nations' General Assembly

Some of the major steps taken by the IAEA with respect to the responsibilities outlined in Article V are

● In 1972, guidelines for "The international observation of PNE under the provisions of NPT and analogous provisions in other international agreements" were developed and approved by the agency's Board of Governors

● In 1974, an advisory group developed "Procedures for the Agency to Use in Responding to Requests for PNE-Related Services" These procedures have also been approved by the Board of Governors

● The Agency has convened a series of technical meetings which reviewed the "state-of-the-art" These meetings were convened in 1970, 1971, 1972 and in January 1975

● Potential PNE Supplier States were approached in December 1974 concerning their participation in elaborating the basic conditions to be incorporated into the special agreements referred to in Article V of NPT

● A separate "Unit for Peaceful Nuclear Explosions Services" was established within the IAEA Secretariat in January 1975

Far from resisting "the accumulation of yet more duties for a secretariat already fully extended", the IAEA has taken active steps to meet its obligations as the appropriate international body mentioned in Article V of the NPT The point that is entirely missed in your editorial is that despite the extensive exchange of information that the IAEA has promoted over several years on this subject (incidentally we do not put out "fairly enthusiastic publicity" but merely publicise the statements of national experts), the equally extensive preparatory work that the IAEA has done on the legal and administrative aspects, not a single formal request for a PNE has yet been received by us We have had a few enquiries from member states, and we have arranged some preliminary investigations, but in no case has a serious project emerged

Finally, your conclusion that someone should have the courage at the NPT Review Conference to suggest that "the treaty be scrapped and new approaches tried" is most difficult to take seriously The NPT and the related Test Ban Treaty are the two major achievements of post-war arms

control negotiations What is needed is to pursue efforts to control the spread of nuclear weapons and to strengthen and supplement NPT by additional non-proliferation nuclear arms control and disarmament measures

Yours faithfully,

SIGVARD EKLUND

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Cabora Bassa hazards

SIR,—The Cabora Bassa Dam, the second barrage across the Zambezi, closed on December 5, 1974 The 171-metres high dam, situated on the Cabora Bassa rapids (the natural boundary between the Middle and Lower Zambezi), will hold back a 250 km long lake, with a mean breadth of 11 km and a maximum capacity of $6\ 23 \times 10^{10}$ cubic metres at a retention level of 336 m AMSL It will inundate 2,739 square km with a mean depth of 25 2 m (maximum depth 157 m) Five south bank turbines at present nearing completion each have a power capacity of 430 MW Four more turbines planned for a future north bank station will bring the eventual power capacity to 3,870 MW

Pre-impoundment surveys of the status of the Zambezi and some of its major tributaries in relation to the dam, began in April 1973 and included physico-chemical characteristics of the rivers (A Hall, I Valente and J da Costa Martins), phytoplankton (J C Oliveira), fish (R Morais), zooplankton and benthos (the author) The objective has been to provide basic data from which to monitor future changes in the valley ecosystem, occurring directly or indirectly as a result of the dam The work has also produced the first information on the Zambezi since Kariba was closed in 1958, and has shown the effect of the combined influence of the Kariba and Kafue dams on the river in Moçambique

In January 1974, a small team studied the probable future impact of the dam on the valley, including such problems as aquatic macrophyte infestation (expected to include *Salvinia molesta* D S Mitchell, *Pistia stratiotes* L and *Eichhornia crassipes* Mart), fisheries development, lake management, the creation of national parks and reserves and the impact of the dam on agriculture, industrial development and existing game reserves and fisheries in the Lower Zambezi Valley During the study, the valley planning authority

engineers, Gabinete do Plano do Zambezi (GPZ) in Tete revealed that they intended to fill the lake to capacity during a period of four months, allowing less than 60 cubic metres of water to discharge daily to the gorge below the dam In normal years, the average flow through the gorge is of the order of 2,000–3,000 cubic metres per second

Recommendations made by the team to the GPZ and the then Portuguese and Moçambique governments included allowance of a minimum discharge from the dam during the filling phase of 400–500 cubic metres per second with the lake taking between one to two years to reach maximum capacity, thus reducing the expected detrimental effects associated with prolonged water reduction in the Lower Zambezi The gorge below the dam, approximately 25 km long (river depth between 30–40 m), has no source of water other than the dam Four major rivers enter the Zambezi between the gorge and the coast, 560 km downstream, namely the Luia, Revuboé, Mazoe (Luenha) and Shire, the Shire draining Lake Malawi to the north Unpublished data supplied by the GPZ shows that these rivers add little by way of volume (though the Shire considerably alters the chemistry of the Zambezi below their confluence) and then only during the latter part of the wet season (mid-January to late March)

Accompanying a press announcement of closure (Noticias de Moçambique, 28 11 74) was the comment that the dam would be completely closed until such time as the water level reached 306 2 m AMSL (approximately three weeks after closure according to the report), after which time water would be discharged into the gorge The lake would then fill at a slower rate The minimum discharge recommendations were ignored The engineers anticipated that little or no noticeable difference in river level would occur during this early filling phase due to the expected onset of the rains This statement contradicts the river flow data supplied by the GPZ and gives no consideration to the possible long-term ecological consequences of such action To compound the problem, local rains did not begin until two to three weeks after closure Within seven days of closure, the river at Tete (approximately 120 km downstream from Cabora Bassa) was reported to have dropped to dry season levels By February 8 this year, levels had dropped sufficiently to prevent operation of water supply intakes for Tete, and no discharge from the dam had taken place up to this date At the same time, large numbers of spawning fish were stranded in the lower river and slaughtered in large numbers by local Africans

This apparent disregard of recom-mendations made with long-term con-siderations in mind bodes ill for future co-operation between ecologist and engineer and could have important long-term repercussions for the agricul-ture (mainly sugar cane), fisheries and game reserves of the Lower Zambezi Valley and its floodplains Important decisions, such as the timing of artificial floods and the controlled fluctuation of water level in order to facilitate de-velopment of hydrophytes, essential for fisheries development in the new lake, must be taken in the near future A dialogue between ecologist and engineer is essential if a balanced use of the resource is to be made

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African finds

Sir,—In his article on the Leakeys' work at East Rudolf (*Nature*, February 20, 1975) Bernard Wood writes, of the Taung discovery in 1924 "It was for-tunate that the remains of a fossil monkey had been recognised at the same quarry only a few months pre-viously " In fact, fossil baboons were found at Taung as early as 1920 On 19th May 1920, a paper on seven or eight fossil baboons from Taung was read in Cape Town before the Royal Society of South Africa by S H Haughton, subsequently Director of the Geological Survey of the Union of South Africa, and still later, Honorary Director of the Bernard Price Institute for Palaeontological Research at the University of the Witwatersrand Haughton's paper of 1920 was not pub-lished in full, though he made his notes available to R A Dart, R Broom and J H S Gear An abstract of Haugh-ton's communication was published in 1925 To accommodate the small baboon skulls from Taung, he had pro-posed to create a new species, *Papio antiquus* However, as his paper was not published and as no type was desig-nated, the name *P antiquus* was both a *nomen dubium* and a *nomen non rite publicatum* Gear recognised that the sample of cercopithecoids from Taung included two different species to which he gave the names, *P africanus* and *P izodi*

Presumably, the "fossil monkey" re-ferred to by Wood was a baboon cranium which, in 1924, one of Dart's students, Josephine Salmons, recovered from the home of E G Izod, a direc-tor of the Northern Lime Company This find led Dart to request R B Young, then professor of geology at the Witwatersrand University and about to survey the Taung area, to speak to the Timeworks manager in the hope of obtaining more specimens

The recovery of the Taung australo-pithecine skull, said by Wood to have been discovered by accident, actually occurred as follows Mr M de Bruyn, a miner at Taung, had been interested for many years in collecting fossilized bones A week before Professor Young's visit, in October 1924, de Bruyn had blasted out a number of fossils These included the brain cast of a baboon and, embedded in another fragment of breccia, a second, larger endocast, among the other rock frag-ments part of a lower jaw was exposed The manager, A E Spiers, and de Bruyn showed the collection to Young, who selected certain pieces which Spiers railed to Dart It was the sec-ond, larger endocast which turned out to be that of *Australopithecus* Another piece of rock made a perfect join with the former and from it Dart extracted the face (There is no evid-ence whatever to support Bernard Wood's statement that "The child's skull was in fact identified by Pro-fessor Young" indeed this would have been virtually impossible since most of the skull was totally embedded in the matrix Only a part of the endocast was showing on the surface of one piece and the back of the lower jaw on another piece Identification was out of the question until Dart had extracted the specimen from its matrix in time for Christmas, 1924)

The article also stated that Taung was situated "in what was then Bechuanaland" This common mistake arose because Taung was situated in the northernmost part of the Cape Province, an area formerly known as 'British Bechuanaland' (and totally dis-tinct from 'Bechuanaland') But this area was in 1924 an integral part of the Union of South Africa, entirely sepa-rate from the Bechuanaland Protec-torate which is today the Republic of Botswana This old name of the nor-thern Cape Province led several scien-tists and textbook writers erroneously to place Taung in 'Bechuanaland' or even 'Botswana'

Wood's statement that ' work has been resumed recently at three of these sites' (in the Transvaal) is mis-leading, since Brain resumed excava-tion at Swartkrans in 1965, while Tobias and Hughes began excavating at Sterkfontein in 1966 Work has con-tinued ever since, in the case of Sterk-fontein without any interruption for 48 weeks a year for over eight years Hence, although the article admirably reflects recent work in East Africa, it does scant justice to the palaeo-anthro-pological researches in the Transvaal over the past decade

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news and views

MOSSBAUER spectroscopy has never achieved the universality of application experienced by many other spectroscopic methods, and will probably never do so This is not due to any intrinsic difficulty in the technique itself, but rather because the number of different elements which can be studied without special facilities is small, and also because the information obtained from the spectra, although frequently crucial to the elucidation of a structure, can rarely be fully interpreted in the absence of other data about the compound concerned But the technique possesses a number of valuable attributes which may be exploited where other analytical methods would fail, and this allows its use in systems which would otherwise pose considerable analytical problems

The basis of Mossbauer spectroscopy is the recoilless emission and resonant absorption of gamma rays of suitable energy The line width of this resonant process is so narrow that the effect can only be demonstrated where the emitting and absorbing atoms are isotopically similar, spectra normally being generated by the Doppler modulation of source or absorber to allow absorption measurements over a small range of photon energies

First observed by Rudolf L Mossbauer in 1957, the effect was rapidly found to have possibilities for the study of changes in the electronic environment of the atoms whose spectrum was measured, because the atoms' environment influenced the nuclear energy levels between which the resonant gamma ray transitions occurred This influence was twofold, and allowed structural information regarding the atomic environment to be obtained

First, any difference between the s-electron density as experienced by the emitting and absorbing nuclei will cause a displacement of the absorption line known as the 'isomer shift' This allows the comparison of s-electron densities in different compounds and thus gives, among other things, information about oxidation states Second, any electron distribution which is not spherically symmetrical may give rise to a 'quadrupole splitting' in which the single absorption line becomes a doublet centering on the original position The separation of the doublet is proportional to the field asymmetry, and provides information on the geometry of the atom's immediate environment In addition, the presence of an internal or external magnetic field may lift the nuclear spin degeneracy and be exhibited as a multi-line spectrum

The letter from Hedges in this issue of *Nature* (page 501) exemplifies the situation where Mossbauer spectro-

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Mössbauer
spectroscopy
in
archaeology

from Nigel J Seeley

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scopy has been used to gain information which would not readily be obtained by other methods Archaeological materials and works of art present many difficulties to the analyst They are frequently complex and unknown mixtures, and in many cases sampling cannot be allowed Provided that the sample geometry is suitable, wholly non-destructive analysis is possible, but unless surface scattering spectra are to be obtained it is generally more convenient to take a sample unless the object is thin, as in the case of a painting

Also, because the Mossbauer spectrum obtained is that of one specific atomic species, the presence of impurities containing other elements, even when present as a major constituent, will not complicate the spectrum, although their attenuation of the gamma rays may be inconvenient This means that the spectrum obtained will be only that of the atomic species being studied, and if that element is present in two or more compounds or environments in the sample a simple and resolvable additive spectrum will be obtained

The elements most suited to Mossbauer studies on archaeological material are iron and tin, although nickel, zinc and gold might be useful in certain circumstances

Iron is not only a particularly convenient element for study, but its very wide occurrence in raw materials results in its presence in a diversity of artefacts Iron impurities in ceramics were first investigated by Cousins and Dharmawardena (*Nature*, **223**, 732, 1969) for typing purposes, and at the recent Symposium on Archaeometry and Archaeological Prospection at Oxford (19–22 March, 1975) Longworth and Warren presented data relating to the black slip on certain samples of Etruscan pottery Here the problem is to identify an iron compound present, with other minerals, in particles too small for conventional X-ray diffraction, but presenting no difficulty regarding the Mossbauer spectrum Though it is not possible to arrive at an identification of an unknown compound from the Mossbauer spectrum alone, comparison with spectra of known likely compounds can readily be used to characterise such materials Glasses and glazes are another class of substance which, having no long-range ordering, are unsuitable for X-ray diffraction, but yield valuable information about the environment of iron atoms present in the network or as microcrystalline impurities

Mossbauer spectroscopy has also been used by Keisch (*Archaeometry*, **15**, 79, 1973) to identify iron-containing pigments on paintings where its non-destructive nature and ability to 'ignore' compounds not containing iron were used to the full Quantitative analysis using Mossbauer spectroscopy is more difficult, at the lattice dynamics of different compounds cause the absorbing atom to vary in its efficiency, but with suitable calibration a degree of quantification can be achieved

There is therefore a number of situations where Mossbauer spectroscopy provides information on archaeological and art materials which is otherwise difficult to obtain, and much useful work remains to be done on glazes and colouring materials Ancient alloys have been little studied from this point of view, and it is likely that such materials as tin bronzes would repay investigation

Nuclear incompressibility

from P E Hodgson

MANY different measurements of the sizes of atomic nuclei have shown that their volumes are closely proportional to the number of nucleons they contain It is thus a very good approximation to assume that the nuclear radius is proportional to $A^{1/3}$, and indeed the relation $R = 1\,25\,A^{1/3} \times 10^{-1/3}$ cm is widely used This result may also be expressed by saying that nuclear matter is incompressible, so that the addition of more nucleons to a nucleus does not increase its density but only its size

But nuclear matter is not infinitely incompressible, and several lines of evidence enable us to estimate its compressibility None of these is very direct, so there is still considerable uncertainty in the values obtained.

In classical physics, incompressibility is measured by the energy needed to reduce the volume (or the density) by a definite amount Since at equilibrium the rate of change of energy with density is zero it is usual to define the incompressibility K by the second differential evaluated at equilibrium radius R

$$K = R^2\, d^2\varepsilon/dr^2$$

evaluated at $r = R = r_0 A^{1/3}$, where ε is the binding energy per nucleon

Using the semi-empirical mass formula and neglecting derivatives of symmetry terms gives

$$K = K_M + K_S A^{-1/3} + 6e^2 Z^2/5 r_0 A^{4/3}$$

where K_M is the nuclear matter incompressibility and K_S the nuclear surface incompressibility This shows that the nuclear incompressibility is a function of A

The incompressibility of nuclei can be estimated indirectly in two ways, one depending on our knowledge of the effective nucleon–nucleon forces and the other on the identification of compression oscillations

One of the most widely used nuclear theories derives from the Hartree self-consistent field theory for atoms In this theory each electron is treated as moving in the field of the nucleus and of all the other electrons. If a reasonable estimate of this field is made, it can be used in the Schrodinger equation to calculate the wave functions of the electrons, and hence to give a more accurate estimate of their field at any point. This calculation is continued until it converges, so that the field coming from the solution of the Schrodinger equation is the same as the field used to solve the equation

Such calculations have been made for nuclei, with appropriate modifications to take account of the absence of a central particle, and the equations are usually solved by electronic computers The complete calculation from the nucleon–nucleon interaction is very complicated, so simpler methods have been developed using effective interactions One of the most successful of these is the Skyrme interaction, and Vautherin and Brink (*Phys Rev*, **C5**, 626, 1972) have used it to calculate a wide range of nuclear properties from the nuclear Hartree equations The charge and matter distributions of a range of nuclei, together with the energies of their single-particle states are given very well, so it is perhaps reasonable to accept the values of other nuclear properties derived from the same interaction, even though they cannot be verified independently

Among these properties is the nuclear incompressibility, and the calculations of Vautherin and Brink gave values of 370 and 342 MeV for the nuclear matter incompressibility calculated from two of their most successful interactions Earlier nuclear matter calculations by Brueckner and Gammel (*Phys Rev*, **109**, 1023, 1958) starting from a phenomenological form of the nucleon–nucleon interaction gave $K = 187$, 167 and 172 MeV for three different interactions, while Campi and Sprung (*Nuclear Physics*, **A194**, 401, 1972) found $K_M = 190$, 213 and 295 for three more forces Statistical calculations by Stocker (*Nuclear Physics*, **A166**, 205, 1971) gave $K = 180$, 230, 195 for three different forces

Pandharipande (*Phys Lett*, **31B**, 635, 1970) has calculated incompressibilities for a range of nuclei using the constrained Thomas–Fermi method and finds values ranging from $K = 123$ MeV for ^{40}Ca and 176 MeV for ^{120}Sn to 190 MeV for ^{208}Pb These values are given by the above expression if $K_M = 266$ MeV and $K_S = -496$ MeV

These calculations with nuclear forces carefully adjusted to fit certain nuclear properties give values of the nuclear matter incompressibility that vary in the range 150–400 MeV This indicates that the incompressibility depends on aspects of the nucleon–nucleon interaction that do not strongly affect the more easily measurable properties of nuclei, so it is difficult to know the reliability of the results obtained

Another approach to the problem of determining the nuclear incompressibility makes use of a particular kind of nuclear oscillation called a 'breathing oscillation' The more familiar types of nuclear vibrations are shape vibrations, like those of a liquid drop or a jelly, and these can take place in an incompressible medium But if the medium is compressible, however slightly, it can oscillate simply by changing its size, while remaining all the time spherical It would look as if it were breathing and hence the name 'breathing mode' for these oscillations

The higher the incompressibility, the higher the energy of the excited nuclear state corresponding to such an oscillation Using a hydrodynamical model (Walecka, *Phys Rev*, **126**, 653, 1962) the energy is given in terms of the incompressibility by

$$E \approx (\hbar^2\, K/m\, r_{R\,MS}^2)^{1/2} \approx 6\,9\,K^{1/2}\,A^{-1/3}$$

(Pandharipande, *Phys Lett*, **31B**, 635, 1970)

It is not easy to identify such states in nuclei They have a monopole vibrational character and so have spin and parity 0^+ and strengths that exhaust a large fraction of the EO sum rule Such states should be readily excited by reactions like inelastic electron scattering One such state was reported by Pittham and colleagues (*Phys Rev Lett*, **33**, 849, 1974) at 8 9 MeV in Pb, but this corresponds to a rather low incompressibility of $K \approx 90$ MeV, so this identification remains uncertain (*Nature*, **253**, 92, 1975)

In oxygen detailed calculations by Sharp and Zamick (*Nuclear Physics*, **A223**, 333, 1974) gave about 28 5 MeV as the energy of the breathing mode state, but it is not yet possible to identify this with an actual state The difficulty of finding the breathing mode states thus prevents them being used to determine the incompressibility at the present time, but the development of more specific ways of exciting these states may eventually make this possible

Breakdown of superfluidity

from a Correspondent

WHAT determines the maximum velocity with which superfluid helium will flow without friction? Can we understand the dissipative processes that occur at supercritical velocities? These are fundamental questions that low-temperature physicists have been asking ever since Landau published his classic theory of the mechanism of superfluidity (*Fiz Zh*, **5**, 71, 1941 and **11**, 91, 1947) A recent paper by Phillips and McClintock (*Phys Rev Lett*, **33**, 1468, 1974), describing observations of the drag on an ion moving at high speed through helium, shows that we still do not have complete answers

The liquid phase of the common isotope of helium, ^{4}He, becomes a superfluid when it is cooled below a temperature of about 2 K The superfluid transition is associated with a form of 'Bose condensation' in the liquid, in which an appreciable fraction of the atoms accumulate in one particular single-particle quantum state (so that all the condensed atoms have, for example, exactly the same momentum) At a finite temperature the superfluid phase behaves like a mixture of two fluids a 'normal' component, behaving

A hundred years ago

IN connection with the recent meeting of the French learned societies, Mr G J Symons writes from Paris as follows —"M Michel threw out a suggestion which appears to me likely to, or at any rate possibly may, be the means of averting the principal source of danger in crossing the Atlantic I refer, of course, to icebergs in foggy weather and the total wrecks which occur from running on to them It is well known that the proximity of icebergs is indicated by a diminution in the temperature of the sea M Michel's proposal is very simple it is merely that Transatlantic steamers should carry a submerged electric thermometer, which might easily be arranged to ring a bell in any part of the vessel on the occurrence of whatever change of temperature might be decided upon "

from *Nature*, 11, 476, April 15, 1875

like an ordinary viscous liquid and composed of thermal excitations in the liquid, and a 'superfluid' component, formed from the background liquid and closely associated with the atoms in the Bose condensate

Suppose that superfluid helium is suddenly set into fairly slow motion along a tube The thermal excitations rapidly come into equilibrium with the tube, and this is observed as a viscous slowing up of the normal component But the velocity of the superfluid component does not change This velocity is identified with the velocity of the condensate, any change in this velocity would imply a change in the velocity of all the atoms in the condensate simultaneously, and this process is believed to occur with negligible probability The superfluid component is therefore observed to flow without friction

Similar considerations apply to the motion of an object through superfluid helium At low speeds it suffers a drag from only the normal fluid Its effect on the condensate is to produce only an adiabatic deformation of the condensate wave friction, corresponding to frictionless potential flow of the superfluid component past the object At very low temperatures, when there is practically no normal fluid, the object suffers negligible total drag

This absence of drag is expected to apply only at sufficiently low velocities As was pointed out, in effect, by Landau, it is energetically favourable for an object that is moving at a velo-

city exceeding a certain ('Landau') critical velocity to create a 'roton', which is one of the possible thermal excitations in the liquid In 1970 E H Takken published a theory of the rate at which rotons can be produced in this way (*Phys Rev*, **A1**, 1220), and showed that an extremely large drag should set in above the Landau critical velocity

In practice the Landau critical velocity for the creation of rotons is hardly ever observed, since another process, the creation of quantised superfluid vortices, usually sets in at a lower velocity The creation of these vortices raises many interesting questions, which have not all been answered, but that is another story The one situation where rotons do seem to be created was discovered by Rayfield (*Phys Rev Lett*, **16**, 934, 1966, *Phys Rev*, **168**, 222, 1968), who showed that negative ions in helium at a high pressure can be accelerated to a limiting velocity that seems to be equal to the critical velocity for roton creation (about 50 m s^{-1})

It is the drag on a moving negative ion at high pressures that has been investigated in detail by Phillips and McClintock They have extended the earlier measurements by Rayfield and others, and they have measured the velocity with which the ions move in a very high electric field They find that the drag on an ion moving at a velocity greater than the roton critical velocity is much less than was predicted by Takken, and they also find some unexpected features in the way in which vortex creation competes with roton creation in the drag process It seems clear that Takken's theory is inadequate, and that we have another example of the fact that even after over thirty years of theoretical effort our understanding of critical velocities in superfluid helium is still far from complete

Lunar duststorms

from David W Hughes

ONE piece of apparatus left on the lunar surface by the Apollo 17 astronauts was a three-axis microparticle detector This was designed to study the cosmic dust environment at the lunar surface to determine the nature and extent of luna ejecta produced by meteorite impacts at other spots on the Moon, to measure possible increases in flux produced by Earth focusing and to look for possible interstellar particles Berg, Richardson, Rhee and Auer (Goddard Space Flight Center, Greenbelt, Maryland) present preliminary results from this experiment in their recent letter to *Geophysical Research Letters* (**1**, 289, 1974) The sensors are

on three sides of a cube, one looking up, the other two looking along the lunar surface, pointing 25° north of east and 25° south of west respectively The cube stands on four legs and is about 20 cm above the level of the lunar soil The mass threshold is typically 10^{-13}g The apparatus is on for 76% of the time, being switched off at the height of lunar day 1,117 particles have been so far recorded during 203 days of observation

One fascinating result from the analysis is that the particle event rate increases when the terminator (the day–night dividing line) passes over the apparatus This increase starts some 40 h before sunrise and ends about 30 h after, the event rate typically increasing by a factor of 100 over the 'non-terminator' rate The sunset effect is less consistent in time of appearance, duration and quality but is definitely present Berg *et al* rule out the direct effect of solar electromagnetic radiation as a cause because the event rate goes up long before the Sun illuminates the lunar region under consideration Particles emanating directly from the Sun can also be discounted because the phenomenon always ends when the Sun is still rising in the sky Rigorous thermal testing of the apparatus prior to launch also rules out temperature effects The authors conclude that they are observing lunar surface particles, moving westward at sunrise and eastward at sunset, just as if a duststorm was sweeping across the lunar surface This storm is along the terminator line, the dust particle moving always in the direction away from the Sun

This could be a perfect example of electrostatic particle levitation and subsequent horizontal soil transport It has been shown that solar ultraviolet radiation will raise the surface of the Moon to a potential of 20 to 40 volts and will give rise to a layer of high-electron density and electric field intensity that extends several centimetres above the lunar surface This field is of the order of 60 V m^{-1} and can easily support small positively charged dust particles Moving them bodily from place to place is another problem which requires some circumstance such as deviation of the potential of dust grains from the median value, this leading to the build-up of repulsive and attractive forces

Dust movement could also be due to variations in the electrical surface charge with position on the lunar surface This idea was first put forward by Criswell (*Geochim Cosmochim Acta*, **3**, 2671, 1972) who suggested that large differences in the electrical surface charge could build up across light–dark boundaries due to the highly resistive nature of the lunar surface If this is the cause of the duststorms the

horizontal variability of the electrical field as a function of solar zenith angle (or distance perpendicular to the terminator) could be inferred from the time variability of impact events obtain from the data of Berg et al The electrical charge of the lunar surface, positive in the Sun and negative out of the Sun, is confirmed by the transport direction of the positively charged dust particles

Confirmation of dust churning in the terminator zone has been obtained from Surveyor spacecraft observations of lunar horizon glow Surveyors 5, 6 and 7 clearly detected a line of light along the lunar western horizon immediately following sunset These observations have been discussed by Rennilson (California Institute of Technology, Pasadena) and Criswell (Lunar Science Institute, Houston) in a recent paper in *The Moon* (10, 121, 1974)

They suggest that the horizon glow is sunlight which has been forward scattered by dust grains ($\sim 10\,\mu m$ in diameter, mass $\sim 10^{-9}$g, ~ 50 grains cm^{-2}) present in a tenuous cloud formed temporarily (duration about 3 h) just above the sharp sunlight–shadow boundary in the terminator zone The grains are thought to be levitated by an electrostatic field of greater than 500 V cm^{-3} across the boundary Rennilson and Criswell also find that the electrostatic dust motion totally dominates the churning produced by micrometeoritic impact The scale height of the glow is between 20 and 30 cm which is of the same order as the height of the micrometeoroid detector at the Apollo 17 site

Rennilson and Criswell comment on the fact that no obvious patterns had been seen by astronauts in the lunar soil, patterns which one would expect to result from the surface dust motion required to produce horizon glow But as horizon glow regions constitute only a small fraction of the lunar surface and tend to occur on slopes (regions avoided by astronauts) this is not too surprising Interestingly the Taurus Littrow region of the Apollo 17 dust experiment is surrounded by mountainous terrain which is ideal for horizon glow production The difference between the duration of horizon glow (3 h) and the micrometeoroid dust storm (70 h) could be entirely due to the different particle sizes responsible, the 10^{-13}g micrometeoroids remaining in the lunar atmosphere much longer than the 10^{-9}g particles responsible for light scattering

It will be most interesting to see if the more detailed analysis of the Apollo 17 site micrometeoroid data reveals storm duration to be particle size dependent When the dust storm had died down the sensor fluxes were found to be notably similar to those

measured in deep space ($\sim 4 \times 10^{-4}$ particles m^{-2} s^{-1} (2π sr)$^{-1}$ This indicates that there is very little normal lunar ejecta with speeds in excess of 0 8 km s^{-1}, the threshold speed of the detectors But the speed of the lunar dust storm particles must exceed this value to have been detected at all, so a certain percentage of them should have speeds in excess of the lunar escape velocity of 2 4 km s^{-1} and should subsequently leave the Moon to join the Solar System dust cloud

Function for *E. coli* penicillin-binding protein

from H R Perkins

NEARLY thirty years ago Duguid proposed that penicillin caused the death of growing bacteria by interfering with the synthesis of their wall components, although at that time the composition and structure of the walls were unknown Since then, as more detailed information about the constitution of bacterial walls has accumulated, progressively more sophisticated explanations of the mode of action of penicillin have become possible

The walls of all penicillin-sensitive organisms contain a structural component called peptidoglycan, which consists of glycan chains of alternating residues of N-acetylglucosamine and its 3-O-D-lactyl ether, N-acetylmuramic acid, to the carboxyl groups of which are joined short peptide chains consisting of alternating L- and D-amino acids An essential feature is that some of these amino acids are trifunctional (for example L-lysine, meso-diaminopimelic acid, or D-glutamic acid) and in the final peptidoglycan, which forms a retaining network round the cell, these amino acids are involved in cross-linking the peptide side chains of one strand of glycan to those of another By 1965 the final cross-linking step of peptidoglycan synthesis had been pinpointed as the target of penicillin action Various cell-free transpeptidation systems are now known to be sensitive to penicillin, and indeed purified soluble *Streptomyces* transpeptidases are inactivated by taking up just one molecule of penicillin or cephalosporin per protein molecule

The amidinopenicillanic acid derivative (FL 1060) described by Lund and Tybring in 1972 acts differently from the β-lactam antibiotics previously known In the first place it is much more active on Gram negative than on Gram positive bacteria, which is the reverse of the situation observed with most penicillins and cephalosporins

Growth of *Escherichia coli*, for instance, is inhibited by 0 02 μg ml^{-1}, and the organism does not respond to this low concentration by developing filamentous forms or 'rabbit's ear' outgrowths that lead to spheroplast production as in the classical response to penicillin On the contrary, the shape of *E coli* treated with FL 1060 is characteristically ovoid, and after a period of time lysis ensues without prior spheroplast formation The second major difference is that even high concentrations of this antibiotic have no inhibitory action on peptidoglycan transpeptidation It seems, therefore, that there must be another point of intervention for FL 1060 apart from peptidoglycan cross-linking, although the change of shape and lack of cell division in *E coli* suggest some connection with cell wall metabolism

The walls of Gram negative bacteria differ from those of Gram positive species in having a much thinner peptidoglycan layer and outside that an outer membrane, which contains lipoprotein and lipopolysaccharide As Braun has shown, a proportion of one particular species of lipoprotein molecule is covalently attached to the peptidoglycan by a peptide linkage Since this linkage is formed after the initial synthesis of peptidoglycan, it may well also involve a transpeptidation reaction that is sensitive to FL 1060, as Park and Burman already speculated

The work of Spratt and Pardee (this issue of *Nature*, page 516) throws new light on the mode of action of amidinopenicillanic acid, and perhaps at the same time on a hitherto unspecified process in cell growth and division They find that in *E coli* all the six binding proteins for radioactively labelled penicillin are in the cytoplasmic membrane Pretreatment of cells with FL 1060 at 5 μg ml^{-1} results in complete blocking of subsequent binding of ^{14}C-benzylpenicillin to only one of the six distinguishable binding proteins and even below the minimum concentration inhibitory for bacterial growth there is considerable competition for binding sites Of a range of penicillins and cephalosporins studied, only the penicillin nucleus 6-aminopenicillanic acid produces the ovoid cells characteristic of the action of FL 1060 and this substance also prevents binding of labelled penicillin to the same specific membrane protein, though to a lesser extent it also decreases binding to some of the other bands

The apparent molecular weight of the specific binding protein is 66,000, and it accounts for only 0 5% of the total binding of ^{14}C-benzylpenicillin Spratt and Pardee reasonably propose that this protein is the target by which the amidinopenicillanic acid affects the
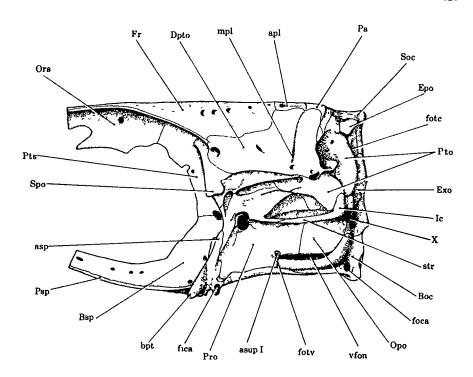
shape of *E coli*, and that it is an enzyme involved in the terminal stages of peptidoglycan metabolism, which "may be specific to Gram negative organisms

There have been many studies of the binding of penicillin to bacterial membranes In bacilli certain carboxypeptidases have been shown to be responsible for some of the observed binding, but many protein species are affected, just as in the absorption of benzylpenicillin by *E coli* The site of emergence of potential spheroplasts from *E coli* under the influence of benzylpenicillin was used by Donachie and Begg in their studies on cell growth, and the relationship of 'penicillinsensitive' sites to the growing bacterium was established It will be interesting to see where, in fact, FL 1060 is absorbed to cells, but presumably this will have to await the availability of the tritiated antibiotic The intriguing similarity of 6-aminopenicillanic acid action suggests that perhaps the binding site requires a positively charged group on the β-lactam molecule, unless, of course, the amidinopenicillanic acid is specifically taken up by the enzyme and then hydrolysed to 6-aminopenicillanic acid The fact that the membrane protein specific for the binding of amidinopenicillanic acid also takes up penicillin implies that the majority of β-lactam antibiotics will probably inhibit its cellular function too, presumably that specific action is masked by the more far-reaching effects on peptidoglycan transpeptidases that these substances also exert In any event, the specific action of FL 1060 should add to the already impressive list of antibiotics that have furthered our understanding of cellular processes

Teleost ancestry, or naming of parts

from B G Gardiner

COMPARATIVE biology is concerned with evolutionary theory and the history of life It is within the second domain that Colin Patterson's monumental paper " The braincase of pholidophorid and leptolepid fishes, with a review of the actinopterygian b r a i n case" (*Phil Trans R Soc*, **269**, 305, 1975) falls In the past hundred years there have been many great comparative works on fishes, notably those of Allis, Jarvik, Nielsen, Stensio and Woodward The use of Hennig's theoretical (phylogenetic) framework however has allowed Patterson to reach more clearly defined objectives than envisaged by his predecessors, while in carrying through the necessary rigorous com-

Patterson's restoration of the neurocranium and attached dermal bones of *Pholidophorus bechi* (from *Phil Tran R Soc*, **269**, 1975)

parative work he has achieved a breadth of study which sets a new standard for the future

Palaeontologists in the past have frequently set out to answer different questions about the history of life In particular they have searched for ancestors and have tried to reconstruct series of ancestor–descendant relationships (for example Chondrostean-Holostean–Teleost) Patterson rejects this approach as unprofitable since it has led to a fruitless search for confirmation of hypotheses Instead his paper shows the great value of the Hennigian framework, which approaches phylogeny through a direct comparison of present-day forms, looking for collateral descendants with a common ancestor (which remains hypothetical) In this way the investigator can formulate testable hypotheses in response to precisely stated problems

Patterson's paper provides the first comprehensive account of the Mesozoic pholidophorid and leptolepid fishes, which are of outstanding interest because they stand in the same relation to living teleosts as do their contemporaries the late Triassic and Jurassic m a m m a l s and 'quasimammals', to living mammals The morphological series he describes illuminates many problems of actinopterygian evolution and ancestry As well as clearing up the history of the cranial fissure and the ossification pattern of the actinopterygian braincase and snout, Patterson confirms the homology between the spiracular groove of primitive actinopterygians (the group

which includes most present-day bony fishes) and the 'prespiracular groove' of rhipidistians (an entirely fossil group) In so doing he rejects the hypothesis that the intercranial joint of rhipidistians and coelacanths is a primitive one related to those between vertebrae and confirms the view of T H Huxley in his Croonian lecture of 1858 in which he said, "It is no more true that the adult skull is a modified vertebral column, than it would be to affirm that the vertebral column is a modified skull"

Patterson has been meticulous in naming of parts, that is in tracing homologous structures throughout his series of fishes and naming them accordingly But, like Huxley, he has realised that the possession of common structures alone does not necessarily lend support to the evolutionary hypothesis unless such structures can be deemed to be 'shared specialisations' of unique origin—found nowhere except in the species under consideration It is in this context that Patterson has analysed the ossification of the braincase and the closure of the cranial fissure with the concomitant displacement of the pterotic by the epioccipital and the loss of the opisthotic and the endochondral portion of the intercalar. The phylogenetic homologies he has demonstrated show the dangers of the topographic approach, which assumes that a bone in a particular position in a series must always be the same bone

Although there are no practical limits to comparative studies, and further development may be confidently predicted, it is fair to conclude that in

a hundred years time Patterson's work will stand much as it does now, a milestone in comparative palaeontology

Palaeontologists keenly anticipate parts 2 and 3 of this work, which Patterson promises on the palate, lower jaws, gills, shoulder girdles and remaining dermal bones

Icelandic layer 3 identified?

from Peter J Smith

THE upwelling of magma to form new lithosphere at oceanic ridges is easy to envisage in general terms, but lack of access prevents any direct investigation of the detailed processes involved In the study of constructive plate margins, the existence of subaerial conditions such as those of Iceland is therefore invaluable, even though by definition such environments are not entirely typical of oceanic ridge zones Indeed, insofar as it may also lie above a rising mantle plume, Iceland itself may be even more atypical than it was considered to be in the days when only a single magma source was envisaged But be that as it may, the few land masses astride ridges offer a unique opportunity of studying an important plate tectonic feature using more traditional geological techniques

G P L Walker began his extensive field studies in Iceland long before any of these ideas were conceived, let alone widely accepted, and over the years he has been able to throw much valuable light on the island's remarkable characteristics But that much still remains to be learned is well illustrated by his latest report (*J Geol Soc*, 131, 143, 1975) in which he draws attention to the significance of a striking geological feature which has until now been largely ignored—the remarkable swarm of inclined basic intrusive sheets which cuts the Tertiary and early Quaternary volcanic pile along the south-east coast

The swarm is impressive by any standards, being exposed over a zone of about 1,700 km² Over a third of that area the sheets comprise more than 10% of the total rock, and over their 100 km² of greatest concentration they account for more than 80% of the total In all, there are tens of thousands of individual sheets with typical thicknesses of 0 2–2 0 m, chilled margins and thickness-related grain size (the thinnest sheets are basaltic, the thickest gabbroic) They increase rapidly in number down the dip, taking the concentration from less than 1% of the total rock at the top to more than 50% within a drop of only a few hundred metres Moreover, the dip angle is itself

Oceanic polarity test fails

from Peter J Smith

IN view of the critical roles of magnetic anomalies and the geomagnetic polarity-time scale as evidence for seafloor spreading, it would be desirable to measure the magnetic polarities of as many oceanic rocks as possible Unfortunately, most of the igneous rocks recovered so far from the ocean floor have been unoriented dredge samples In some cases the tops of pillow lavas may be recognised, and this limited orientation is sufficient to allow polarity to be determined by conventional palaeomagnetic methods But for the majority of samples for which this procedure is not possible, Irving (*Can J Earth Sci*, 5, 1319, 1968) devised a method of determining polarity which involved comparison of the coercive force spectrum of a rock's natural remanent magnetisation with that of an artificially induced anhysteretic moment

As applied by Irving to five Cainozoic basalt samples of known orientation, the method seemed to work And in a similar test, Brooke *et al* (*Can J Earth Sci*, 7, 1515, 1970) obtained a success rate of 11 out of 12 But in a thorough study by Mohajer-Ashjai (*Can J Earth Sci*, 12, 62, 1975) on 20 lavas from Oregon and Mull, agreement between known and predicted polarities has been obtained in only 10 cases Not surprisingly, Mohajer-Ashjai concludes that the Irving technique is not reliable enough Although it is not completely worthless (since the probability of getting a 50% agreement purely by chance is less than 0 054) it seems that unoriented dredge samples will be of little use as a source of magnetic information about the oceanic crust

closely related to the concentration of sheets, being less than 10° where sheets are scarce or absent but increasing to 35°–50° where they are most abundant Lastly, and significantly, the direction in which the sheets dip is downwards towards the spreading axis—the same direction as the less steeply dipping country rocks

How did this remarkable swarm originate? The answer, Walker argues, is by a very simple mechanism involving density contrasts between the Icelandic crust and the uprising magma As basaltic magma rises, its density changes because of the onset of vesiculation caused by the exsolution of dissolved

gases Generally, this change (decrease) will take place at depths less than 1 km, but different batches of magma with different volatile or phenocryst contents and different chemical compositions will begin to decrease in density at slightly different levels within this range

The presence of these variations raises the possibility that under certain circumstances different batches of magma may behave in quite different ways as they move up through the crust For example, the variations could be critical if the density–depth profiles for the magma were close to that for the solid crust, which seems to be the case in the upper 4 km Below a depth of about 4 km, the density of all the magma is undoubtedly lower than that of the crust, and so the magma will rise in that region In the upper 4 km, however, where the magmatic and crustal density profiles are comparable, two possibilities arise If the density of the magma never anywhere rises above that of the crust, the magma will continue its ascent until it reaches the surface and eruption results On the other hand, if the magma density does rise above the crustal density at some point, the vertical progress of the magma will be there arrested

Walker's proposal is that some of the magma does one thing and some does the other In particular, the magma that reaches a level at which its density equals that of the surrounding crust then ceases to rise and, instead, moves away laterally along an equi-density surface as an intrusive sheet An obvious difficulty with this view is that in practice the solidified intrusive sheets are not actually horizontal but are inclined away from the spreading centre with an inclination that decreases up the dip However, there are several factors which could account for equi-density surfaces of variable slope, including a non-even distribution of dykes across the width of the active zone, temperature variations in the country rock and an infilling of the voids in the country rock by zeolites in certain areas All three could contribute to density variations in the crust, although the dyke distribution is thought to be the most important In addition, however, as the magma sheet rises up-slope its density will decrease, especially if vesicles begin to form, and so it may actually cross equi-density surfaces and move towards crustal rocks of lower density

According to Walker, therefore, the existence of the swarm of inclined basic intrusive sheets may be explained in terms of processes at the spreading axis, albeit modified by the particular crustal environment through which the axis passes But if that is the case, why are such sheets limited to this particular

part of Iceland? Perhaps they are not In Walker's view, the sheets are only exposed in this part of Iceland because the erosion here has been particularly severe He proposes that the sheet swarm actually "constitutes a widespread and integral part of the structure of Iceland and is not merely a local phenomenon" Elsewhere it is not seen because it lies below sea level

He then goes on to identify this widespread swarm with crustal layer 3 Of the four main crustal layers identified seismically beneath Iceland, layers 0, 1 and 2 have been interpreted reasonably as basalt lavas and associated rocks But the nature of layer 3, which has an average thickness of 6 km and begins at an average depth of 3 5–4 0 km, is still unknown Certainly a layer 3 of basic intrusive material should have the right P wave velocity (6 35 km s^{-1}) The next step should therefore be to test Walker's hypothesis by measuring seismic velocities within the sheet swarm in south-east Iceland It is unfortunate that all previous seismic profiles were run outside the swarm or in parts of it where the concentration of sheets is very low

Group selection

from Robert M May

MANY animals seem to exhibit altruistic behaviour, whereby individuals sacrifice their own interests for the good of the group The 'warning cries' uttered by some birds are an example the individual puts his own life at greater risk for the benefit of his fellows Many other apparent examples are to be found in the patterns of behaviour with which animals regulate their population density

But natural selection acts on individuals, not groups It has been argued that 'group selection' (the evolution of traits which are advantageous at the group level, but disadvantageous at the individual level) cannot take place within the classical Darwinian framework

In many cases, closer study reveals that seemingly altruistic behaviour directly benefits the individual as well as his group, thus removing any paradox In other cases, group selection can be seen to derive from kin selection the altruist's actions are of direct advantage to his relatives, and therefore his gene type is favoured by his action, even if his own life is not As W D Hamilton has pointed out, social insects probably exhibit extreme examples of kin selection In the Hymenoptera, females are more closely related to their sisters than to their daughters, and so from a genetic point of view they do better to care for a sister than

to devote an equal amount of care to their own offspring

There remain, however, examples of apparent group selection which cannot be evaded by the above arguments Maynard Smith, Levins, Boorman and Levitt and others have tried to construct mathematical models which exhibit the phenomenon These models envisage a population distributed over many islands or other such environmental patches, with occasional migration between patches They seek to show that when a 'selfish gene' appears it will (by classical individual selection) take over the local population, but that the consequent disadvantage to this group as a whole may cause its extinction before the selfish gene can spread to the other populations All these models require the most delicate tuning if the desired effect is to be exhibited, and none of them constitutes a truly plausible explanation for group selection

Recently, two different models have been proposed, both of which exhibit group selection in a robust fashion

The first of these was suggested by May, and has independently been elaborated at monograph length by Gilpin (*Group Selection in Predator–Prey Communities*, Princeton University Press, 1975) These models take advantage of specifically nonlinear aspects of predator–prey or plant–herbivore interactions, which can produce stable oscillatory patterns in population density Small advantages to the selfish individual can now be nonlinearly magnified into disastrously large amplitudes of population oscillation for his group The result is that group selection can be rapid and powerful, although selfish genes continue to appear by mutation, the groups in which they appear are quickly extinguished before they spread

A simpler and more compelling mechanism has been pointed out by Wilson (*Proc natn Acad Sci U S A*, 72, 143–146, 1975) As in essentially all these models, first suppose the total population is constituted of several isolated groups Periodically these populations are mixed together and then redispersed (One may think of the annual episodes of winged dispersal among so many insects) The population contains two genotypes altruists, *a*, and selfish non-altruists, *b* Wilson notes that it can be that in each group the altruists suffer higher mortality than the non-altruists, but that nevertheless the overall proportion of altruists in the population can increase from generation to generation

This statistical paradox is familiar in other areas, for example medical biometrics, and is best illustrated with a numerical example To fix ideas, let *a* be birds which give warning cries,

thereby decreasing the average mortality in their group at the expense of increasing their own mortality rate relative to the selfish *b* Suppose we have two patches the first containing 25*a* and 75*b*, the second 75*a* and 25*b* Initially *a* therefore constitutes 50% of the population The birds in the first patch suffer higher mortality by virtue of the smaller complement of altruists, just before the next dispersal phase there are, say, 30 individuals, 6*a* and 24*b* The second group does better, coming down to, say, 70 individuals, 50*a* and 20*b* Note that in both groups the fraction of altruists, *a*, has decreased (from 25% to 20% in the first, and from 75% to 71% in the second), but that the altruistic genotype has increased in the population as a whole (from 50% to 56%)

Wilson's model has the quality of *a posteriori* obviousness which characterises so much of the best in science It illustrates group selection, pure and simple

Emplacement of intrusions

from G M Brown

THE geological evolution of the British Isles is a long and complex story but one period is particularly well understood, due to a prodigious amount of scientific investigation since the 1920s Relatively recently in geological time, about 60 million years ago in the early Tertiary, active volcanoes erupted in what is now north-west Britain Vast outpourings of basaltic lava were succeeded by the emplacement of a very complex array of igneous bodies in the form of dykes, cone sheets, ring dykes and other intrusive masses The rock types are variable but include, predominantly, the products of medium to slow crystallisation of basaltic and granitic magmas The intrusive complexes are believed to represent generally the eroded cores of central-type volcanoes that developed, as in Iceland, on and within the plateaux of the earlier flood lavas Remnants of the flood lavas are preserved particularly well in Antrim but also in the Scottish Inner Hebrides The later, central volcanic complexes constitute the bulk of many of the Hebridean islands such as Arran, Rhum, Mull and Skye, as well as Ardnamurchan (mainland Scotland) and the Mournes and Carlingford mountain regions in northern Ireland

The volcanic regions have been mapped in ever-increasing detail, including geophysical measurement, by British geologists, and a host of laboratory studies has been conducted on the phase petrology, mineralogy and

geochemistry aimed at understanding the genesis of the rock suites Yet the mechanism of emplacement of the intrusive complexes has remained a major problem Walker (*J Geol Soc Lond*, **131**, 121, 1975) has now provided a first, really new and comprehensive explanation since the earlier hypotheses of Bailey, Richey, Harker and associates (chiefly 1904–1936) Those hypotheses were based chiefly on the concept that some intrusions were facilitated by pressures exerted upwards by underlying magma bodies while others were formed during the release of such pressures and the downward collapse of crustal blocks The mechanisms were quantified by reference to Anderson's classic work (1936) on the dynamics of formation of cone-sheets (upward pressure), ring dykes and cauldron subsidences (downward collapse) Since then, refinement of Anderson's hypothesis rather than an alternative hypothesis has been the characteristic of modern, sparse work on the problem

Walker's paper, in his own words, " presents no new geological facts but sifts some of the great volume of existing data " The data are drawn from the meticulous observations and superb geological maps produced by the earlier Geological Survey staff referred to From that, and his own observations in Iceland, he proposes radically that the main control of emplacement mechanics was the uprise of granitic diapirs in the region, early in the history of each Tertiary intrusive centre The Goatfell granite body in northern Arran is quoted as a good example 'of such a diapir, but other examples are described Granitic liquid would have a low magma density of about 2 3, and an analysis is made of the relationships between hydrostatic (magma) pressures and lithostatic (load) pressures in the vicinity of such diapirs The construction of isopiestic (equal pressure) surfaces, after the manner of Bradley (1965), results in models that are convincing when related to the forms of intrusions in the volcanic centres, and to the early-published evidence for early doming and annular folding at most centres

Basaltic magmas, associated with the main volcanic outpourings would, according to Walker, generate granitic (acid) magmas below the surface to the point at which a large body of granitic magma develops, rises as a diapir, and grows during ascent (100–1,000 Km³) Basaltic magma would be channelled into a magma trap at its base and develop into a cylindrical prism of basic intrusives as the diapir rises The granitic body would release effusive magmas when it reached the surface, and thus diminish in size, while the basic rocks would rise in its wake

View from Venus
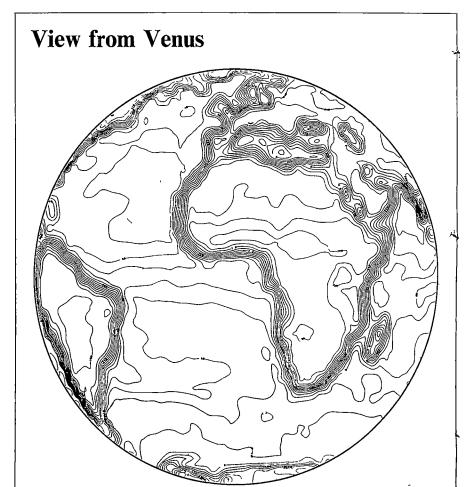
Image corresponding to 0 h The contour interval is 10 K with every other contour labelled From Webster, Chang, Darby and Finkelstein, *Icarus*, **24**, 144, 1975

RADIO observations of the planets are all very well, but they would be better still if we had a benchmark against which to judge them Webster *et al* have now provided such a benchmark by using Nimbus 5 observations at 19 35 GHz to produce a "degraded and smoothed" simulation of what the Earth would look like to a radio astronomer on Venus armed with an instrument with a resolving power of 1″ The clear boundary between land and sea would, it seems, make it possible to determine very accurately the rate of rotation of the Earth, and also show up large permanent features (see figure) On this evidence and that obtained from radio studies of Mars and Venus, the Earth is undoubtedly the most interesting of the inner planets

Other batches of magma would be diverted to zones outside the diapiric zones, where excess hydrostatic pressures existed (the model explains cone-sheet formation in this way) Late subsidence of the cylinder of basic intrusives below the diapir could provide for outward dipping structures such as ring-dyke and bell-jar (curved flange) intrusives

The hypothesis is difficult to fault on the basis of a vast and otherwise embarrassing array of good quality, earlier field observational data It is welcome because Anderson's model has long been found wanting, especially in regard to the role of his postulated ring-dyke mechanics The need, now, is for a more rigorous consideration of

Walker's model, especially from a possible refinement of the density data and a more detailed analysis of pressure relations and diapiric movement applied to mobile granitic magmas Certain assertions in the paper, peripheral to the main argument but nevertheless relevant, are not immediately acceptable Walker refers to the Rhum ultrabasic rocks as representing ultrabasic magma (not necessarily so), and does not face up to the sequence of events in central Skye (granitic complexes later, rather than earlier than the Cuillins layered basic intrusives) He postulates a development of igneous layering parallel to the inclined floors of gabbro intrusions of "confluent cone-sheet type" whereas such layering, if con-

trolled by gravitational crystal settling, as he accepts it, is independent of a pre-existing floor slope Some pertinent, recent publications on the Ardnamurchan cone sheets and the Skye hybrid (marscoite) bodies are not referred to Early tidying-up is a natural requisite for an hypothesis that will need severe testing

The mechanics of emplacement is a local problem, whereas the genesis of the British Tertiary magmas relates to universal problems Work over the past decade or so has concentrated on the latter, and yet the origin of the granitic magmas remains in dispute Most petrological, experimental and isotopic studies favour an origin by remelting of the crustal rocks (anatexis) rather than by crystal fractionation of the basaltic magmas Walker's model leaves little doubt that only crustal remelting is acceptable, yet surprisingly he does not refer to that clue or to magma genesis in general His granitic diapirs are shown as developing before the underlying cylinder of basic intrusives has crystallised, which would be impossible if they were late fractions of a solidifying basic intrusive, quite apart from the fact that only a very small volume of granite is produced by the latter process Proponents of the fractionation hypothesis will not approve of Walker's granitic diapirs, which makes it all the more important that his hypothesis be explored to the full, and consideration be given to the several broader petrogenetic implications

Silicon wafers without sawing

from Andrew Holmes-Siedle

ONE annoying deficiency in semiconductor device technology today is the limitation in size of the single-crystal ingots of semiconductors (such as silicon) which can be grown Nearly all mass-produced ingots are grown by slow pulling from the melt (the Czochralski method) The best ingots that can be produced in this way are about two inches in diameter and six inches long Thus, in silicon device factories, devices are formed on thin wafers cut from such ingots in a direction perpendicular to the long axis, like slices of sausage Neither the size nor shape are conducive to good yield of devices in large production runs of transistors, integrated circuits or other 'small-signal devices'

For the simpler solar cell diode, area is the dominant factor Indeed, a square foot would be a good area for a single cell, such an area just cannot be obtained at the moment, even by the expensive method of cutting ingots along the long axis, as in sawing a

plank Instead, an array of little cells has to be wired together, which greatly increases the cost and lowers the reliability of solar panels

For these reasons, crystal growers have for a long time been exploring ways of making silicon crystals of larger area which might eliminate wasteful and arduous sawing and polishing One technique which has recently raised hopes could be regarded as two-dimensional pulling of crystals Crystallisation proceeds along a thin film of molten silicon lying in a mould The method can be turned into a flow process if the film can be generated continuously A ribbon, of theoretically unlimited length could then be produced Tyco Laboratories, of Boston, have been exploring this process, termed edge-defined film-fed growth (EFG) As yet, few of the ribbons produced are single-crystal, most consist of large polycrystals which themselves contain imperfections in the form of dense tangles of dislocations Nevertheless, there is often a dominance of one crystal orientation at the surface It is thus possible that, under the right conditions, one might be able to promote totally single-crystal growth in a layer of silicon deposited on to the ribbon by one of the known epitaxial growth techniques

Now one epitaxial growth technique is already widely used to improve the properties of integrated circuits formed on ingot-grown silicon wafers This is the so-called chemical vapour deposition method The layers grown are several micrometres thick and are of excellent single-crystal quality The advantage of this deposition method is that only flowing gases are involved and the method, again a flow process, can probably be scaled up to cover large areas

Thus, the process for making large, though imperfect silicon wafers in one step can possibly be married to this already widespread epitaxial growth technique to form a production method which yields large ribbons of crystalline silicon (it should be noted here that, for most active semiconductor devices, all of the electronic functions occur in a layer a few micrometres thick at one surface only)

Henry Kressel and co-workers at RCA Laboratories are the first to report this marriage In *Applied Physics Letters* (25, 197, 1974) they note the happy result that, when the epitaxial growth from the vapour is carried out on ribbons of p-type silicon, made by Tyco Laboratories using the EFG technique, the defects in the original wafer are not strongly propagated into the newly forming crystalline lattice This is shown clearly in X-ray topographs but more to the point is the finding that p–n diodes

with good minority-carrier lifetime, good breakdown voltages and low leakage currents can be made by putting down first p-type and then n-type layers on to this silicon ribbon Although the ribbon used by the investigators were only 1 3 cm wide, there is no basic bar to scaling this up by an order of magnitude Ribbbon lengths of 2 metres, and widths of 2 5 cm have been reported unofficially

For the integrated-circuit and transistor manufacturer, the availability of such ribbons could give the era of single-crystal devices a new lease of life, maintaining the price-competitiveness of this form against the rapidly developing and currently less expensive thin-film device fabrication methods

For the solar array designers, the prospect of cheaper, more reliable solar panels is improved if the ribbon method succeeds Indeed, it is probably no coincidence that the work at RCA took place under the supervision of Paul Rappaport, one of the pioneers of the silicon photovoltaic device and co-author of a recent survey for the National Academy of Sciences on the terrestrial use of solar converters The latest news over the agency wires is that, despite some remaining problems with crystallinity, preparations are being made to make ribbon silicon on a factory scale

Chromosomal proteins and control of transcription

from a Correspondent

> The Florida Colloquium on Molecular Biology "Chromosomal Proteins and Their Role in the Regulation of Gene Expression" was held at the University of Florida in Gainesville on March 13 and 14

ALTHOUGH there have been many reports correlating changes in non-histone chromatin proteins with alterations in gene expression, there has to date been little direct evidence linking the two processes Encouraging however were reports from two laboratories of direct demonstrations that nonhistone chromatin proteins mediate control of transcription of the histone and globin genes G S Stein (University of Florida, Gainesville) has synthesised a DNA complementary to histone mRNAs and used it to establish that the transcription of histone genes from chromatin is restricted to the S phase of the cell cycle Chromatin re-

construction experiments showed that the nonhistone proteins of S phase chromatin are responsible for regulating the transcription of histone mRNA sequences R S Gilmour and J Paul (Beatson Institute for Cancer Research, Glasgow) have provided evidence using reconstruction experiments that in chromatin of mouse foetal liver cells and DMSO-stimulated Friend cells nonhistone proteins render globin genes transcribable In both the histone and globin transcription studies the potential interference of endogenous RNA was ruled out There has been some concern about both chromatin reconstitution and the use of bacterial polymerases for chromatin transcription, but the histone and globin systems at least seem amenable to these approaches

Several laboratories are engaged in isolating and characterising defined nonhistone chromatin protein fractions and exploring their functions H Busch (Baylor College of Medicine, Houston) has been isolating chromatin and nucleolar proteins by differential salt extraction and fractionating them on a preparative scale by two-dimensional polyacrylamide gel electrophoresis His group has purified and is at present sequencing a protein whose appearance is correlated with nucleolar activation

Another technique which has been much used for fractionation of chromatin proteins is DNA affinity chromatography V Allfrey (Rockefeller University, New York) described new methods for the fractionation of nuclear nonhistone proteins which utilised DNA of various average C_0t values covalently attached to solid supports such as amino-ethyl sepharose, Sephadex G25 and Sephadex G200 This method yields reproducible fractions some of which interact preferentially with 'moderately reiterated' sequences while others bind to unique DNA sequences Some of the DNA binding proteins exhibit a high and selective affinity for cyclic AMP or cyclic GMP G Patel (University of Georgia, Athens) described a class of proteins which do not exhibit species-specific binding but rather display a preference for AT-rich and single-stranded DNA T Y Wang (State University of New York, Buffalo) reported the isolation of two nonhistone protein fractions from *Ehrlich ascites* tumour chromatin, one which activates and the other which suppresses transcription of DNA *in vitro* The action of each of these protein fractions requires the homologous RNA polymerase II and homologous DNA The inhibitory protein, which is electrophoretically homogeneous, blocks RNA synthesis at initiation prior to formation of the first phosphodiester bond

There seemed to be a general consensus that DNA binding fractions are enriched in phosphoproteins Though DNA affinity chromatography appears to be an effective method for fractionation of chromatin proteins, caution must be exercised in concluding that *in vitro* binding reflects biological activity

T C Spelsberg (Mayo Clinic, Rochester) discussed the specificity of the binding of progesterone–receptor to hen oviduct nuclei and chromatin Although multiple levels of binding were observed in all tissues examined, the highest affinity binding was only detected in chromatin from target tissues The removal of one nonhistone protein fraction 'AP3' resulted in loss of high affinity binding

For some time phosphorylation of chromatin proteins has been correlated with the activation of transcription in a variety of biological systems Consistent with these observations, studies by R Platz and L S Hnilica (University of Texas, Houston) indicated that during sea urchin development the rate and specificity of nonhistone protein phosphorylation reflects the process of differentiation

L J Kleinsmith (University of Michigan, Ann Arbor) described the properties of two principal nuclear components of the chromatin protein phosphorylation mechanism Twelve kinase activities were identified, several being cyclic AMP-dependent and others refractory to or even inhibited by cyclic nucleotides Nuclear phosphatase activity was also described Dr Kleinsmith, with G Stein and J Stein, has also found that enzymatic dephosphorylation of nonhistone chromatin proteins from S phase HeLa cells resulted in a reduction in chromatin template activity and in the transcription of histone mRNA sequences The latter studies provide evidence for a functional relationship between phosphorylation of nonhistone chromatin proteins and RNA transcription

● Histones were not entirely overlooked at the colloquium R Chalkley (University of Iowa) addressed the problem of deposition of preexisting and newly synthesised histones on replicating DNA He showed that in contrast to DNA, which is replicated in a semiconservative fashion, histones are randomly distributed between the preexisting and newly replicated DNA strands

T Langan (University of Colorado, Denver) presented evidence that there are two types of F_1 histone phosphorylation, which can be distinguished on the basis of the specific amino acid residue phosphorylated and the requirement for cyclic AMP Phosphorylation of serine 37 takes place in non-

proliferating cells following hormonal or cyclic AMP stimulation Phosphorylation of amino acid residues other than serine 37 is strongly correlated with the rate of cell growth and is unaffected by cyclic nucleotides Distinct enzymes which catalyse the two types of F_1 phosphorylation reactions have been isolated P Byvoet (University of Florida, Gainesville) discussed the enigmatic phenomenon of histone methylation and its possible role in the maintenance of chromatin structure He showed that agents which are capable of intercalating into the DNA double helix increased the extent of histone methylation

● The last portion of the colloquium was concerned with the arrangement of proteins on DNA in chromatin B Sollner-Webb and G Felsenfeld (NIH, Bethesda) have examined the structure of chromatin, both purified and within nuclei, by digestion with *staphylococcus* nuclease The kinetics of nuclease digestion are consistent with the "beads on a string" arrangement of chromatin Sollner-Webb and Felsenfeld also presented evidence which suggests that the organisation of proteins in the neighbourhood of globin genes differs between reticulocyte chromatin, which actively transcribes globin mRNA, and erythrocyte chromatin where globin transcription is largely repressed In reticulocyte chromatin a distinct segment of the globin gene is not accessible to poly-D-lysine binding, presumably because it is covered by chromatin proteins In erythrocyte chromatin the entire globin sequences seem to be present in both poly-D-lysine accessible and inaccessible regions J Gottesfeld and J Bonner (California Institute of Technology, Pasadena) described a method for isolating a template-active chromatin fraction by digestion with DNase II The DNA of this fraction contained a subset of the total genetic sequences which is partially tissue-specific The template-active fraction has a decreased histone content, totally lacks histone I, and is enriched in nonhistone protein Limit digests of total chromatin with DNase II revealed nucleoprotein bodies equivalent to those described by Sollner-Webb and Felsenfeld But limit digests of the template-active fraction did not yield these nucleohistone particles

articles

Mantle deformation beneath the South African and Siberian platforms

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Xenoliths of mantle lherzolite from kimberlite pipes in Africa and Siberian Yakutia have undergone deformation and thermal excitation of some of their constituent minerals Their deformation states support the view that the mantle deforms systematically by creep processes resulting from shearing stress initiated, perhaps, during plate movement

XENOLITHS thought to represent material from the deeper parts of the upper mantle are brought to the surface in kimberlite magmas The most frequently occurring xenoliths are of lherzolite (olivine + ortho and clinopyroxene ± pyrope garnet), harzburgite (olivine + ortho-pyroxene ± pyrope garnet), eclogite (garnet + soda–alumina rich clinopyroxene), and pyroxenite Less frequent, or rare, xenoliths are the kyanite and corundum eclogites, and grospydites which contain a garnet rich in calcium, plus pyroxene and kyanite[1-6]

Lherzolite xenoliths from the kimberlite pipes in Africa provide evidence of deformation of the Earth's mantle[7-15], they fall into broadly-defined textural categories

Granular xenoliths of medium to coarse grain-size (1–5 mm), with weak microscopic fabrics, in which olivine and pyroxene show various strain effects, including undulose extinction, microfractures, kink and deformation bands and lamellae, slip-planes, and subgrain development, contain normal calcic-

diopsides that seemingly re-equilibrated at temperatures between 900 and 1,100 °C and at depths between 100 and 150 km (refs 7–10)

Xenoliths with variable microscopic fabric patterns containing porphyroclasts seem to have suffered increasingly intense shearing stresses through progressive deformation proceeding through a combination of creep (especially in olivine) and subgrain development The diopsides in these lherzolites become increasingly subcalcic with deformation and seem to have re-equilibrated at temperatures between 1,100 °C and 1,400 °C at depths between 150 km and 190 km (refs 7–10) The most intensely deformed of these lherzolites grade into types with a distinctively striped foliation, and they contain sparsely distributed relic porphyroclasts of partly granulated pyroxene with porphyroclasts of undeformed pyrope garnet

I have examined deformed lherzolites from African kimberlites contained in the Imperial College collection, similar lherzolites in the collection of A Nicholas (Nantes University), and xenoliths from the Siberian Yakutia kimberlite pipes, in the collection of N Sobolev

These comparative studies of deformed lherzolites indicate that similar types of lherzolite from the deeper parts of the upper mantle in widely separated regions have had similar deformation histories Compositional data on the minerals from these lherzolites also indicate that their thermal states may be comparable

Table 1 Some analyses of coexisting Pyroxenes from lherzolites of Udachnaya Pipe, Yakutia* (see ref 6)

	Yd 5		Yd 18		Yd 161		Yd 92		Yd 111		Yd 40	
	Diop	Enst	Diop	Enst	Diop	Enst	Diop	Enst	Diop	Enst	Diop	Enst
SiO_2	55 5	58 5	54 9	58 6	55 7	58 0	54 9	58 4	54 5	59 0	54 4	58 8
TiO_2	0 11	0 05	0 26	0 19	0 26	0 15	0 04	0 04	0 00	—	0 41	0 17
Al_2O_3	1 11	0 65	1 47	0 54	1 23	0 50	0 82	0 46	1 01	0 33	1 57	0 61
Cr_2O_3	1 10	0 29	2 00	0 43	1 98	0 50	1 08	0 36	1 08	0 25	0 88	0 27
FeO	3 07	4 95	3 32	5 33	3 25	5 50	2 96	4 93	1 67	4 64	3 12	5 53
MnO	0 11	—	—	0 13	0 05	0 13	—	0 08	0 00	0 13	0 07	0 11
MgO	19 5	35 8	18 4	35 6	19 4	34 7	19 5	35 4	17 7	36 3	18 1	35 5
CaO	17 5	1 03	16 9	0 86	16 8	0 80	19 2	0 85	21 6	0 39	17 3	0 67
Na_2O	0 98	0 17	1 66	0 24	1 5	0 29	0 91	0 12	0 84	0 04	1 68	0 19
K_2O	0 04	—	—	—	0 04	—	—	—	0 08	—	0 05	—
Total	99 02	101 44	98 91	101 92	100 21	100 60	99 31	100 64	98 51	101 08	97 58	101 85

Cations (on basis of 6 oxygen atoms)

	Yd 5		Yd 18		Yd 161		Yd 92		Yd 111		Yd 40	
Si	2 009	1 979	1 994	1 978	1 994	1 985	1 992	1 991	1 996	1 996	2 001	1 985
Al (IV)	—	0 013	0 006	0 011	0 006	0 010	0 008	0 009	0 004	0 004	—	0 012
Ti	0 002	0 002	0 004	0 006	0 009	0 004	0 001	0 001	—	—	0 011	0 004
Al (VI)	0 048	0 013	0 059	0 011	0 046	0 010	0 027	0 009	0 040	0 009	0 071	0 012
Cr	0 030	0 008	0 057	0 011	0 056	0 014	0 031	0 010	0 031	0 006	0 027	0 007
Fe^{2+}	0 094	0 140	0 100	0 150	0 099	0 158	0 089	0 139	0 051	0 130	0 095	0 156
Mn	0 002	—	—	0 003	0 002	0 003	—	0 002	—	0 003	0 002	0 003
Mg	1 052	1 804	0 995	1 791	1 035	1 771	1 048	1 798	0 966	1 830	0 993	1 784
Ca	0 678	0 037	0 657	0 031	0 645	0 029	0 745	0 031	0 847	0 014	0 681	0 024
Na	0 070	0 011	0 118	0 016	0 103	0 019	0 065	0 008	0 062	0 002	0 119	0 013

	Yd 5	Yd 18	Yd 161	Yd 92	Yd 111	Yd 40
Ca/(Ca+Mg)	39 2	39 8	38 4	41 6	46 7	40 7

Petrography of deformed lherzolites

I will describe two lherzolites which are considered to be typical of the deformed varieties one from the Jagersfontein (South Africa) kimberlite, and one from the Udachnaya kimberlite pipe in Siberian Yakutia

In the lherzolite from Jagersfontein porphyroclasts of enstatite and diopside have been drawn out into lozenge and spindle shaped, partly granulated relicts and, together with undeformed porphyroclasts of pyrope garnet, they are set in a granoblastic-polygonal[15-18] matrix of olivine grains 100 µm or larger in size Small relict porphyroclasts of pyroxene merge outwards into irregularly shaped sub-grains with diffuse boundaries and finally (particularly in enstatite), into outer zones of polygonal grains many of which have triple-point junctions, these grains are of the order of 25 µm or less in size Narrow zones of fine-grained enstatite run from the apices of the granulated relict porphyroclasts into the olivine matrix, which they cut sharply These zones of crystals, which resemble stripes, give the most deformed lherzolites the foliation that typifies hand specimens, they seem to have been produced by a late, intensive, shearing during which stresses have been taken up by pyroxene rather than by the already deformed olivine The lherzolite Ud.5 (Yd 5 in Table 1) comes from the Udachnaya pipe which is located in the Daldyn–Alakit region of Siberian Yakutia north of the Arctic Circle and near the edge of the Siberian platform[6] About 70 thin sections of some 40 lherzolite xenoliths from the Udachnaya pipe were examined About 75 % of these were deformed, the rest had coarse-grained, undeformed textures[7-10,15]

Ud 5 is typical of the deformed varieties and shows an early stage in the development of the striped foliation It contains a few relict olivine porphyroclasts, together with elongate or lozenge-shaped porphyroclasts of partly granulated enstatite and diopside, and undeformed pyrope garnet These porphyroclasts are set in an olivine matrix that has a granoblastic-polygonal texture and an average grain size between 70 and 100 µm

The larger subgrains in the partly granulated enstatite have diffuse boundaries and merge into the relict porphyroclast, the small (25–30 µm), polygonal, grains are furthest away from the relict grain Some zones of matrix olivine are separated from each other by the stripes, and some of the matrix olivine grains have flattened tops as though they were sheared off, or the grains changed shape by diffusion of material away from points of stress[19] Both the Jagersfontein and the Udachnaya lherzolite xenoliths have textures transitional to the most intensely deformed lherzolites described from African kimberlite pipes[11,13]

Thermal history and origin

The pyroxenes in deformed or sheared lherzolites from African kimberlites have re-equilibrated at high temperatures and pressures[7-10], the greater the degree of deformation the higher the temperature and pressure Since many of the Udachnaya lherzolites show comparable degrees and types of deformation it is clearly important to determine whether their pyroxenes also re-equilibrated under high P/T conditions

The temperature of re-equilibration of diopside that coexists with enstatite can be determined if the diopside contains low molecular proportions of ferrosilite and jadeite In these circumstances re-equilibration temperatures can be obtained by applying the experimentally determined temperatures for compositions on the diopside–enstatite solvus at a pressure of 30 kbar to the Ca/(Ca+Mg) ratios of the natural minerals[9,10,16] Using this method for several diopsides from the Udachnaya lherzolites (analyses in ref 6, selected examples in Table 1) gives re-equilibration temperatures between 1,130 and 1,250 °C for minerals from the deformed xenoliths, and a low temperature of 950 °C for diopside (Yd 111 in Table 1) from a granular
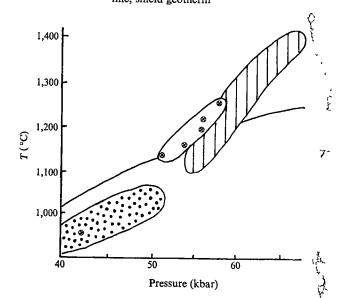
one These temperatures compare with those obtained for diopsides from deformed and undeformed African lherzolite xenoliths[7-10]

To estimate pressures of re-equilibration, and therefore depths of origin of the lherzolites, it is necessary to know or Al_2O_3 content of the enstatite that coexists with the diopside and pyrope garnet Then, the experimental data on the solubility of Al_2O_3 in enstatite at different temperatures and pressures can be used[17], after adjustment has been made to allow for Al_2O_3 combined with Na_2O in the jadeite molecule. Unfortunately data are not yet available on the pressures at which the solubility of Al_2O_3 in enstatite falls below 1 %

A few analyses of coexisting diopside–enstatite pairs from the Udachnaya garnet-bearing lherzolites are available (Table 1), and they show that a major problem in applying the experimental data on Al_2O_3 solubility in enstatite at different pressures is that the Udachnaya enstatites contain <1 % Al_2O_3 These amounts are reduced still further if allowance is made for Al_2O_3 combined with Na_2O in jadeite But it seems reasonable to assume that the pressures at which the Udachnaya enstatites re-equilibrated were probably not less than those defined at various temperatures by the 1 % Al_2O_3 isopleth in the experimental system[17], and the latter has been used in conjunction with the re-equilibration temperatures from the coexisting diopsides to provide rough approximations of the pressures This gives re-equilibration pressures of 50–59 kbar (or depths of origin between approximately 165 and 180 km) for the deformed lherzolites, and 42 kbar (depth of origin approximately 130 km) for the granular variety Again, these figures compare with those for the African lherzolite xenoliths

In Fig 1 the data for the pyroxenes from Udachnaya lherzolites have been plotted in a way similar to that used for the African lherzolite xenoliths[7-10] Accepting that compositional problems make the plots of the Udachnaya data slightly less accurate than those for the African material, two important features are still apparent First, the data for the Udachnaya lherzolite xenoliths plot close to or in similar fields to the data for the African xenoliths Second, the re-equilibration temperatures of diopsides from the deformed lherzolite xenoliths of both Udachnaya and African kimberlite pipes rise sharply with increasing pressure and both the trends are oblique to that for the shield geotherm In effect, diopsides from both sets of deformed lherzolite xenoliths are thermally excited and their re-equilibration temperatures apparently lie along a geotherm referred to as 'perturbed'[9]

Fig. 1 Udachnaya pyroxene data Stippled, diopsides from 'granular' lherzolites (Africa)[9], hatched, diopsides from 'sheared' lherzolites (Africa)[9], Udachnaya diopsides[6], solid line, shield geotherm

Further implications

Accepting that the observed deformation of the thermally excited lherzolites took place in the mantle it is possible, from ? petrographic and chemical data, to make some general observations on the state of lherzolite assemblages below a depth of 150 km in the mantle below the African and Siberian platforms

First, deformation of the lherzolites seems to be systematic, increases with depth of origin of the kimberlite magmas to at least 190 km and apparently proceeds through a combination of creep and subgrain formation The deformation is accompanied by thermal excitation of the pyroxenes that coexist with pyrope garnet and olivine in these assemblages Minor compositional differences in constituent minerals and therefore in the bulk lherzolite assemblages, for example like those that exist between the minerals of the Siberian Yakutia and African xenoliths[6-10], do not significantly affect the general correlation between thermal and deformation states in lherzolites at depth (Fig 1)

Second, the constituent minerals in the lherzolites deform in a systematic fashion[11,12,15] and in a particular order Olivine deforms more easily, and at an earlier stage, than enstatite, which seems to deform a little more readily than diopside Pyrope garnet usually remains undeformed to depths of at least 190 km, though slight granulation of the mineral has been observed in one or two highly deformed lherzolites

Third, there seem to be limits to the grain sizes attained by individual minerals during deformation For example in early stages of deformation of the lherzolites, matrix olivine grains may be of the order of 300 μm or a little less[11], but in later stages, when enstatite and diopside have commenced granulation, matrix olivine grains may be of the order of 50–100 μm Olivine grains smaller than 40 μm are infrequent in xenoliths I have examined Enstatite forms small (25–35 μm) grains, during early stages of its deformation, but in the most deformed lherzolites enstatite grains as small as 10 μm have been observed[11,15] Enstatite, or diopside, grains smaller than this do not seem to have been described

Fourth, there is no evidence of lherzolites in which diopside has re-equilibrated at temperatures higher than 1,450 °C (refs 9 and 10 and Fig 1), although this may in part be a function of unavailability of material from depths much greater than 200 km I note, however, that the T/P gradient for deformed lherzolites in Fig 1 is much steeper than that of the shield geotherm, and it may be that further stress at depths > 200 km could increase temperatures to the point where small volumes of partial melt were produced in the lherzolites, rather than further deformation and mineral re-equilibration

A major problem in geology at the moment is to account for the correlation between deformation states of lherzolite xenoliths from depths 150–190 km and the fact that P/T re-equilibration data for their pyroxenes plot along a 'perturbed' geotherm (Fig 1) It has been suggested that this perturbation of the geotherm took place during plate movement when Gondwanaland fragmented during the Mesozoic, the resulting shearing stresses causing deformation and frictional heating in the lherzolite assemblages at depth[9] A difficulty in totally accepting this view at the moment is that xenolith-bearing kimberlites from Africa and Siberia range in age from pre-Cambrian to post-Cretaceous and we do not know if deformation is restricted to lherzolite xenoliths that give Mesozoic dates The age of the Udachnaya kimberlite pipe in Yakutia is unfortunately uncertain with K/A determinations on phlogopite and olivine giving dates ranging from upper Palaeozoic to late Jurassic respectively[21] But it is clear that only a general, and major, event (or series of such events) could have produced comparable textures and thermal states in lherzolites from widely separated regions in the Earth's mantle It may be, as some geologists now believe, that plate movement has been operative since the Precambrian and that deformed lherzolites provide a record of the way the mantle has reacted to stress over a long period of time

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The chemistry of sea salt aerosol and its measurement

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The chemistry of precipitation (rain and snow) has been extensively monitored since the 1950s, with the analysis performed for nine ions in solution It has been suggested that this monitoring should be extended to include the analysis for heavy metals The examination of results which we report here indicate that routine measurement of one of the most common ions, sodium, has been of too low a quality to prove the most significant current theory about its behaviour in the atmosphere

OF the nine ions which are measured[1]—SO_4^{2-}, Cl^-, NO_3^-, NH_4^+, K^+, Mg^{2+}, Ca^{2+} and H^+—only sodium and chloride are closely related in atmospheric occurrence in that the major source of both is the sea They occur in seawater in a Cl-Na ratio of 1 80 by weight, which is signified by R_s in this paper If there were no other sources, and no separation of the two ions in samples of sea salt aerosol, their weight ratio would always be R_s, whatever the degree of dilution Other land sources of chloride[2,3] and sodium[4] have been suggested but not proven to be of any significance in precipitation chemistry The most substantial and widely
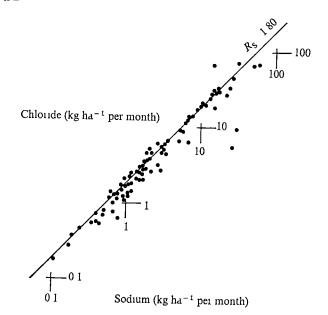
Fig. 1 Monthly amounts of chloride and sodium in precipitation at three coastal stations of the European Air Chemistry Network in southern Sweden and Norway, 23, 38 and 53, for the period 1960–62 The observed values of the Cl–Na weight ratio fall close to the ratio in seawater, 1 80, which is signified by R_s Deviations from R_s are distributed similarly among months with large and small amounts of salt in precipitation

accepted, if controversial, theory about sea salt in the atmosphere is the Cauer hypothesis[5] which states that the sea salt chloride and sodium are separated by chemical or photochemical action in the air and that, from pure sea salt origins, the ratio of chloride to sodium in precipitation, signified by R, can range below and above R_s Early measurements in Sweden[6] seemed to bear out this theory but a re-examination of the data has suggested that the principal support for the theory, the large deficit of chloride in precipitation near the coast, resulted from incorrect chemical analysis[7]

Discrepancies in monitoring data

An examination of monitoring data has shown discrepancies across national boundaries in Europe, similar to discrepancies in the calcium–magnesium ratio which have been reported elsewhere[8] In the UK there is a discrepancy between results obtained by different laboratories The dominance of sea salt as a source allows us to identify one laboratory as being most probably correct A characteristic of the results from the other laboratory is found in data from monitoring networks in other parts of the world, suggesting that their results should be critically re-examined Figure 1 shows the monthly amounts of sodium and chloride at several coastal stations in Norway and Sweden over a period of 3 yr Deviations from R_s are equally distributed among months where the amounts of salt were large and small In considering the distribution of the deviations from R_s we will disregard the absolute amounts of salt and treat only the value of R We will consider the distribution in time of deviations and determine whether the observed distribution could arise from geochemical phenomena or from laboratory factors

Where deviations from R_s result from phenomena which modify the sea salt while it is suspended as an aerosol, the same phenomena should affect samples at adjacent stations in the same month A phenomenon which causes a high value of R at European Air Chemistry Network (EACN) stations 23, 38 and 53 in southern Norway and Sweden should also produce high values at stations 69, 70 and 71 in Denmark Whether it would also affect station 54 in northern Norway is a matter for conjecture A laboratory cause for a set of high values could be

either the dispatch of a contaminated set of bottles to the stations or a deviation in the analysis for either sodium or chloride when a month's samples have been returned If it is a rare event it is unlikely to coincide with a similar event another laboratory We have selected the period 1959–63 f analysis because, for the first half of that time the Danish samples were analysed in Copenhagen with samples from 12 other Danish stations The Norwegian and Swedish samples were analysed in Stockholm The Danish analysis was discontinued in May 1961 After August 1961 the samples from Danish stations 69, 70 and 71 were analysed in Stockholm together with the samples from about 30 Norwegian and Swedish stations We can thus compare, for the same set of stations, the degree of coincidence of high or low values of R when analysed at either one or two laboratories

Table 1 shows the occurrence of values of R less than 1 50 and greater than 2 16 during the 60-month period Samples were collected at each of the seven stations in 44 months, during which time the Danish samples were analysed in Copenhagen for the first 21 months The results are expressed in the contingency tables (Table 2) which give the number of occasions on which none, one, two, three or four of the four Swedish and Norwegian stations experienced low (or high) values of R tabulated against the simultaneous occurrence of

Table 1 Cl–Na ratios in precipitation

	Norway and Sweden				Denmark		
	23	38	53	54	69	70	71
1959	•	•	•	•	•	•	•
	•	•	+	•	•	•	•
	•	•	+	+	•	+	−
	•	−	•	•	•	−	•
	•	•	•	•	•	•	•
	−	−	•	−	•	•	•
	−	•	−	•	•	−	•
	•	•	•	+	•	•	•
	•	•	•	−	−	−	−
	•	•	+	−	+	•	•
1960	•	•	•	•	•	•	•
	•	•	•	•	•	−	−
	−	−	•	−	•	•	−
	+	•	•	•	−	•	•
	•	−	−	−	•	+	+
	•	•	•	•	−	−	−
1961	•	•	•	−	•	•	+
	•	•	•	•	•	+	+
	•	•	•	•	•	−	−
	•	•	•	•	•	•	−
	−	−	•	•	•	•	•
	+	+	•	+	+	•	+
	+	•	−	•	+	•	−
	−	•	−	−	•	−	−
1962	−	−	−	•	−	−	−
	•	•	−	•	•	•	•
	•	•	•	•	•	•	•
	•	−	−	•	−	−	•
	−	−	−	•	−	−	•
	+	•	+	+	+	•	•
	•	•	•	•	•	•	•
	•	−	•	−	•	•	•
	•	•	•	•	•	•	•
1963	−	−	−	−	•	•	−
	•	•	+	•	•	•	•
	•	−	+	−	•	•	•
	•	−	•	•	•	•	−
	•	−	•	•	•	•	•
	•	−	−	•	•	•	•
	•	•	+	•	•	•	•
	+	+	+	•	+	+	+
	+	+	+	•	•	+	+

−, Indicates Cl–Na less than 1 50, •, indicates Cl–Na between 1 50 and 2 16, +, indicates Cl–Na greater than 2 16

Table 2 Contingency tables

(a) (i) Low values (R < 1 50)

3 Danish

		0	1	2	3	
4	0	6	1	3	1	11
Norwegian	1	3	2		1	6
and	2		1			1
Swedish	3	2	1			3
	4					0
		11	5	3	2	21

(ii) High values (R > 2 16)

3 Danish

		0	1	2	3	
4	0	13	1	2		16
Norwegian	1	3	1			4
and	2		1			1
Swedish	3					
	4					
		16	3	2		21

(b) (i) Low values (R < 1 50)

3 Danish

		0	1	2	3	
4	0	9	1			10
Norwegian	1	2	2			4
and	2	4		1		5
Swedish	3			1	1	2
	4		1	1		2
		15	4	3	1	23

(ii) High values (R > 2 16)

3 Danish

		0	1	2	3	
4	0	16				16
Norwegian	1	2	1			3
and	2					0
Swedish	3		1	2	1	4
	4					0
		18	2	2	1	23

low (or high) values at none, one, two or three Danish stations Table 2a covers the 21 months during which two laboratories operated Table 2b covers the period when all samples were analysed in Stockholm

Table 2 shows that the two laboratories produce low (and high) values of R at different times In the first period, when the analysis was carried out in separate laboratories, on none of the three occasions on which three or more of the Stockholm results fell below 1 50 did more than one Danish result fall below 1 50 From Table 2a (i), we see that of the five occasions on which two or three of the Danish stations recorded low results, on none of these did more than one Swedish or Norwegian station record a low result Similarly with the occurrence of high values, in Table 2a (ii), although the situation is less clear in this as only thirteen high values were recorded at the seven stations in 21 months

In the second period, when all samples were analysed in Stockholm, a clear association appeared between high (or low) values in Denmark and high (or low) values in Sweden and Norway Table 2b (i) shows that on four occasions low values were recorded for two or more stations in each section of the network, and b (ii) shows that on three occasions high values were recorded at at least two stations in each section of the network

What does this tell us about the records of the Network? There is a factor, unrelated to the chemistry of sea salt aerosol or precipitation, which leads occasionally to the finding of Cl–Na ratios in precipitation which deviate significantly from the sea salt ratio If we can take the simultaneous presence of three or four deviant values out of a possible four as evidence of its occurrence then the results of most of the Norwegian and Swedish stations are affected by it one month in four In assessing the significance of the factor to the records of the EACN we must consider not the number of occasions on which it occurs but the number of observations which are changed by the factor The occurrence of three low values of R

in Denmark and three in Norway and Sweden n one month, counted as a single occurrence of the factor in the contingency tables above, will affect the records as much as single occurrences of low values of R on six different months Seen in this light Table 2b (i) and (ii) indicates the following score for seven stations during 23 months (Table 3)

If this interpretation is accepted then it seems that at least 23% of the coastal precipitation sample analyses in Stockholm deviate significantly from R_s because of the factor Not more than 17% deviate from R_s because of other causes Thus, there is no strong evidence for a deviation from the sea salt ratio as a result of natural causes such as have been proposed by Rossby and Egnér[6] or by Eriksson[9] If any such deviation does occur at coastal stations it is so subtle as to be masked by an artefact of the network which we believe to be a laboratory factor

In the UK, where precipitation sampling and chemical analysis has been performed since 1957 by the Meteorological Office in the traditional EACN way, a second programme has been commenced by the Atomic Energy Research Establishment (AERE) using neutron activation, X-ray fluorescence and other techniques which differ from those used by the EACN The AERE results which we quote here have been taken from a report which was published in 1972[10] The AERE monthly results for Wraymires, in Cumbria, in 1971 are plotted on Fig 2a with Cl and Na on logarithmic scales In all months R falls close to R_s When the excess Cl, or Na, is estimated for each month the total of each for the year is a 3% excess of Cl and a 5% excess of Na, which are barely greater than the measurement errors At the EACN station at Eskdalemuir, for which the monthly amounts of Cl and Na are shown in Figure 2b, there were no months with excess Cl The excess Na amounted to 97% of the Na from a presumed sea salt source It would not be unreasonable to assume that at Eskdalemuir, 110 km north of Wraymires, there is a and source which, throughout the year, contributes as much sodium to the precipitation as does the sea Such an assumption s not borne out by an examination of the Lerwick results for the same period

At Lerwick, which is a maritime site receiving ten times as much sea salt as Eskdalemuir, there is also a great excess of sodium, as seen in Fig 2b Could this come from a local land source? The excess amounts to 25% of the sea salt sodium over the year and in total quantity is 2 5 times the excess at Eskdalemuir It would require a very strong land source of sodium in the Shetlands for such an observation to be real But if there were a Shetlands source it would have to operate in sympathy with that near Eskdalemuir because the two stations experience high, and low, values of R in the same months Figure 2b shows that in 1971 the low values of R occurred at both stations between April and September It is most unlikely that so many common factors relate the chemistry of precipitation at Lerwick and at Eskdalemuir, which are 550 km apart and very differently exposed to sea and land sources, while Eskdalemuir and Wraymires, which are 110 km apart and similarly exposed, have little in common if the data is all to be believed The significant factor relating Lerwick and Eskdalemuir is that samples from both are analysed in the one laboratory Yet it is the Wraymires results, from a different laboratory using quite different analytical methods, which conform to the most reasonable expectation about the

Table 3

	Number	%
Total number of observations	161	
Observations of R between 1 50 and 2 16	96	60
Low values attributable to the factor	21	13
Low values not necessarily attributable	20	12
High values attributable to the factor	16	10
High values not necessarily attributable	8	5
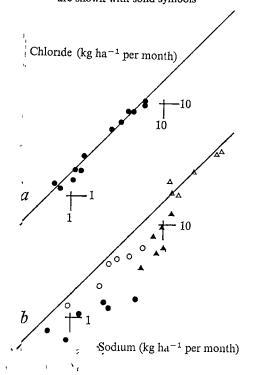
chemistry ofprecipitation This is that the predominant source of both chloride and sodium is the sea, and that these two ions remain associated during their transport on the wind in just the way they are associated in seawater It seems that the system which produces the EACN results should be examined to discover thecause of the incorrect sodium or chloride analyses J L Browrscombe and M S Porter (Meteorological Office, private communication) report that the phenomenon to which B C Oddie referred has recurred in some but not all of the later years ind that it does not occur in all of the three rain ranges nowoperating at Lerwick

The UK is just one of a number of countries which are participating in the EACN Although there are minor local variations, ill laboratories are using a similar set of analytical methods Only in the UK can we be reasonably certain about the deficiencies of the EACN precipitation analysis We would like, thougl, to point out certain similarities in results from other countries which might indicate that similar deficiencies exist there

In Fig 3a the annual total of Cl and Na are plotted for a number of UK stations for the years 1960–62 The most maritime station, Lerwick, receives an apparently small fractional excess of sodium and the inland stations, including Eskdalemuir, receive larger fractional excesses That exces was less in 1960–62 than in 1971, also shown in Fig 3a, the year for which we have compared Eskdalemuir and Wraymires But the previous section of this paper has demonstrated that the excess at Eskdalemuir is almost certainly illusory On common-sense grounds, and the basis of the experience at Scandinavian coastal stations which were discussed at the beginning of this paper, there is even more reason to believe that the excess sodium at Lerwick is illusory Thus, the fractional deviations from R_s, which are largest where the amount of salt is least, seem to be generated by deficiencies in the system But the UK is not the only country to record such characteristics In Fig 3b are plotted for various countries he annual amounts of chloride and sodium for several stations in the years noted on the diagram It is evident that in broad terms the results from each of these are similar to those for the UK This is not indisputable proof that there are deficiencies in analysis in each of these countries But before the

Fig. 2 For 1971, the monthly amounts of Cl and Na in precipitation recorded a, by the AERE at Wraymires (●), b, by the Meteordogical Office at Eskdalemuir (O, ●) and at Lerwick(△, ▲) In b the months between April and September are shown with solid symbols
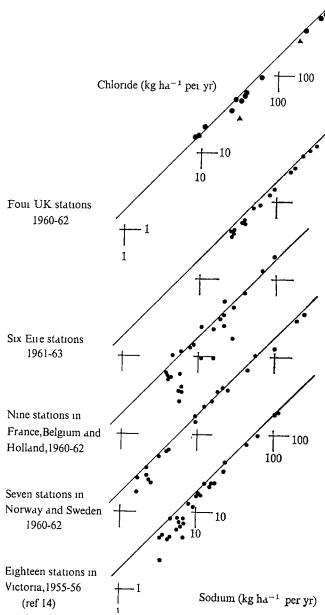
Fig. 3 Annual amounts of chloride and sodium in precipitation at a number of stations of the EACN in various countries, and in Victoria, Australia In each country there is a tendency for excess sodium to occur, the fractional excess over sea salt being greater where salt amounts are least

excess sodium is advanced as evidence of either land sources of sodium or chemical processes in the air transforming the sea salt aerosol, as for example that by Oddie[11], the possibility that it is an artefact of the system of chemical analysis should be considered Before any further monitoring is done such artefacts should be eliminated

There is an increasing awareness that data quality control programmes must be built into routine monitoring of the environment[12,13] The EACN experience with sodium and chloride analysis should alert us to one hazard of such programmes if all laboratories cooperating in a programme of sample exchange are using the same analytical methods, or perhaps the same sample bottles, there is a possibility that all are recording the same bias, thereby allaying justified fears about the various possible forms of interference with results While the internal consistency of data from coastal stations has given us a sufficient test when sea salt is plentiful, it was only the availability of a novel analysis which allowed us to determine that sea salt is the principal source of both sodium and chloride in precipitation at some inland stations

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Paracoccus denitrificans and the evolutionary origin of the mitochondrion

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It is demonstrated that Paracoccus denitrificans *resembles a mitochondrion more closely than do other bacteria, in that it effectively assembles in a single organism those features of the mitochondrial respiratory chain and oxidative phosphorylation which are otherwise distributed at random among most other aerobic bacteria A feasible evolutionary transition from the plasma membrane of an ancestral aerobic bacterium resembling* P denitrificans *to the inner mitochondrial membrane is suggested*

ACCORDING to the endosymbiotic theory of the evolutionary origin of the eukaryotic cell, as developed by Margulis[1], the mitochondrion has evolved from a free-living prokaryote resembling a present-day aerobic bacterium, which had been taken up by a plastid-free, amoeboid cell, the protoeukaryote, that depended on fermentation for the production of ATP In time the prokaryote suffered a progressive loss of autonomy, its proliferation kept pace with the cell division of its host, many of its biosynthetic capabilities were lost or taken over by its host, and its metabolism became integrated with that of its host

The endosymbiotic theory implies that the outer mitochondrial membrane is derived from the protoeukaryote, and that the inner mitochondrial membrane and its invaginations (the cristae) are homologous with the plasma membrane of present-day bacteria Here we describe the modifications of the bacterial plasma membrane which would be necessitated by the evolutionary transition from a free-living, aerobic bacterium resembling *Paracoccus denitrificans* (previously *Micrococcus denitrificans*[2]) to a mitochondrion In this transition the ATPase and the constitutive components of the respiratory chain are retained, the adaptive components of the respiratory chain are lost, and the transport properties of the plasma membrane are altered so that, in its new role as the inner mitochondrial membrane, it is able to integrate the metabolism of the organelle with that of the surrounding cell We chose *P denitrificans* as a plausible ancestor because, when all possible biochemical parameters are compared, it resembles a mitochondrion much more closely than do other aerobic bacteria This comparison is summarised below and will be presented in detail elsewhere[3]

Comparison

Those mitochondrial features which *P denitrificans* possesses, and which have a widespread (or perhaps universal) distribution among those bacteria which can make a respiratory chain, include nicotinamide dinucleotide trans-

hydrogenase[4,5], NADH and succinate dehydrogenases[6-8], and the tricarboxylic acid cycle[9,10]

Those mitochondrial features which *P denitrificans* possesses, but which have a limited distribution among other bacteria, include phosphatidyl choline as a major constituent of the membrane phospholipid fraction[11,12], straight-chain saturated and unsaturated fatty acids accounting for nearly all the membrane fatty acids[11,13,14], ubiquinone-10 as the sole functional quinone of the respiratory chain[6,15,16], two *b*-type and two *c*-type cytochromes as easily distinguishable components of the respiratory chain of aerobically grown cells[16-19], cytochrome *aa₃* as the cytochrome oxidase[6,16,20], and a sensitivity to low concentrations of antimycin A and rotenone, both of which are inhibitors of mitochondrial electron transport[16,21] (see Fig 1) Furthermore, mitochondrial cytochrome *c* more closely resembles the soluble cytochrome *c* isolated from *P denitrificans* than the *c*-type cytochromes isolated from other bacteria, in both physical and structural properties[22-24] The soluble cytochrome *c* of *P denitrificans* is unusual among bacterial *c*-type cytochromes in that it is to a large extent interchangeable with mitochondrial cytochrome *c* in its ability to react with mitochondrial cytochrome oxidase[22,25-27] and with the two cytochrome oxidases of *P denitrificans*, that is, the constitutive cytochrome *aa₃*[27,28] and the inducible cytochrome *cd*, which functions *in vivo* as a nitrite reductase[28,29]

Furthermore, *P denitrificans*, like *Hydrogenomonas eutropha*[31] but unlike *Escherichia coli*[32] and *Bacillus megaterium*[33], resembles a mitochondrion[34,35] in the stoichiometry of oxidative phosphorylation as indicated by measuring H⁺/O ratios Phosphorylating particles prepared from the plasma membrane of *P denitrificans* show a mitochondrial type of respiratory control[36,37] that is rarely observed in other phosphorylating bacterial preparations[8,38] As in mitochondria the respiratory rate is increased by the addition of ADP (respiratory control), or by the addition of carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) (an uncoupler of oxidative phosphorylation) and decreased by the addition of venturicidin which, like oligomycin, inhibits ATPase[39] (Fig 2)

We should like to emphasise that although none of these mitochondrial features is unique to *P denitrificans* no other species of bacterium possesses as many[3] *Rhodopseudomonas spheroides*, however, when grown aerobically in the dark has a mitochondrial type of respiratory chain[19], but the stoichiometry of oxidative phosphorylation and the presence of respiratory control are not known Future research will probably reveal that *P denitrificans* is not unique in the degree to which it resembles a mitochondrion, and it will then be viewed as a representative of a small group of aerobic bacteria (probably including *Rps spheroides*) all having an obvious affinity with the mitochondrion

In addition to the mitochondrial features found in *P*
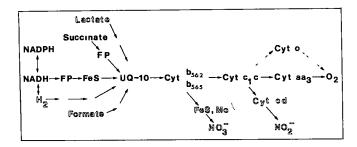
Fig. 1 The respiratory chain of mitochondrion and of *P denitrificans* Components of the mitochondrial respiratory chain, and of the constitutive respiratory chain of *P denitrificans* are in heavy print, the additional adaptive components of the respiratory chain of *P denitrificans* are in lighter print Cytochrome *o* has been found in the mitochondria of only a few organisms[68], and its significance in the constitutive respiratory chain of *P denitrificans* is not known From ref 3

denitrificans described above, there seems to be no significant feature of the mitochondrial respiratory chain or ATPase which is present in another bacterium, but known to be absent from *P denitrificans*

Most of the differences between the respiratory chains of a mitochondrion and *P denitrificans* can be accounted for by the presence in *P denitrificans* of adaptive components which are absent from mitochondria These adaptive components may be viewed as *ad hoc* additions to the constitutive portion of the respiratory chain, which is itself essentially similar to the mitochondrial respiratory chain (Fig 1) The constitutive components of the respiratory chain of *P denitrificans* are nicotinamide nucleotide transhydrogenase, NADH and succinate dehydrogenases, flavoprotein, iron–sulphur proteins, ubiquinone-10, cytochromes of the *b*-type and *c*-type, and cytochrome *aa₃* The adaptive components are hydrogenase, formate and lactate dehydrogenases, nitrate reductase, and nitrite reductase (cytochrome *cd*) (Fig 1)

In their criticism of the endosymbiotic theory, Raff and Mahler[40] pointed out that, although aerobic bacteria possess cytochrome respiratory chains similar in function to mitochondrial cytochromes, "there are significant differences which suggest a considerable evolutionary divergence between mitochondrial and bacterial cytochromes" The differences cited are (1) the insensitivity of bacterial electron transport systems to some of the generally used inhibitors of mitochondrial electron transport, (2) the greater variety of bacterial cytochromes, (3) the poor cross reactivity of mitochondrial cytochrome *c* with most bacterial cytochrome oxidases, and vice versa, and (4) the dissimilarities in the primary sequence, redox potential and isoelectric point of bacterial and mitochondrial *c*-type cytochromes We have noted above that while differences (1), (3) and (4) may apply to bacteria in general, these differences do not apply to *P denitrificans* and *Rps spheroides* since in these respects these bacteria resemble the mitochondrion while most other aerobic bacteria do not Furthermore, it is shown below that the presence in *P denitrificans* of "bacterial" cytochromes in addition to "mitochondrial" cytochromes raises no serious objection to an evolutionary transition from an aerobic bacterium resembling *P denitrificans* to a mitochondrion, since these "bacterial" cytochromes are readily dispensed with under the appropriate environmental conditions

Hypothetical evolutionary transition

A hypothetical transition from an aerobic bacterium resembling *P denitrificans* to a mitochondrion, as envisaged by the endosymbiotic theory[1], would involve the loss of the adaptive components and the retention of the constitutive components of the respiratory chain of *P denitrificans*

(Fig 1) The synthesis of the adaptive components in *P denitrificans* is readily suppressed in the presence of alternative electron donors or electron acceptors that are "more acceptable" Hydrogenase, for example, is present only when cells are grown in the presence of hydrogen and in the absence of organic compounds such as glucose[41], and nitrate reductase is absent from cells grown aerobically in the presence of nitrate[42] Similarly cells of *P denitrificans* grown anaerobically in the presence of nitrate can use either nitrate or oxygen as terminal electron acceptors but, when both are supplied, use oxygen in preference to nitrate[3,43]

The postulated evolutionary transition does not require the adoption of new features by the constitutive respiratory chain of *P denitrificans*, although modification of some components may occur The limited extent of such changes is clear from X-ray structure analyses[24] of the readily solubilised *c*-type cytochromes of *P denitrificans* and of the mitochondrion The indications are that there is a very considerable similarity in the amino acid sequences of the two cytochromes[24] The exact extent of this similarity will be revealed by the detailed amino acid sequencing now being carried out (E Margoliash, personal communication)

The similarity in the H^+/O ratios observed with *P denitrificans* and with the mitochondrion imply that the respiratory chain is arranged in a similar way in the plasma membrane of *P denitrificans* and in the inner mitochondrial membrane The ATPases are also essentially similar in their mode of operation in *P denitrificans* and in a mitochondrion[44], suggesting that the process of oxidative phosphorylation need not be altered during the evolution of the mitochondrion

So far, the proposed evolutionary transition from an aerobic bacterium resembling *P denitrificans* has not necessitated the acquisition by its plasma membrane of any entirely new features, nor has it involved a significant alteration in the mode of operation of those features already present When the respective transport systems of *P denitrificans* and a mitochondrion are compared, however, it becomes apparent that there are mitochondrial features not found in bacteria, such as *P denitrificans*, as well as bacterial features not found in mitochondria Furthermore, some carriers operate in a different way in bacteria and mitochondria
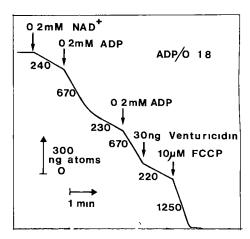
Fig 2 Mitochondrial type of respiratory control in particles prepared from *P denitrificans* Membrane particles were prepared by a slight modification of the procedure of John and Hamilton[37] The reaction mixture contained, in a total volume of 3 ml 30 μmol Tris-acetate (pH 7 3), 15 μmol magnesium acetate, 30 μl ethanol, 0 1 mg alcohol dehydrogenase (Sigma A7011), and 0 54 mg particle protein Further additions were made as indicated Oxygen uptake was measured in a Clark-type oxygen electrode at 30 °C The ADP/O ratio was calculated as described for mitochondria by Chance and Williams[69] The numbers alongside the traces refer to the rates of oxygen uptake in ng atoms per min per mg protein
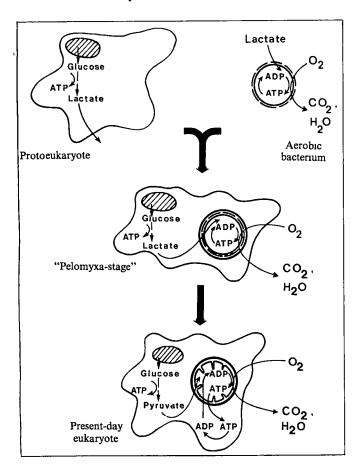
Fig 3 Hypothetical evolutionary transition from a free-living aerobic bacterium to a mitochondrion A fermenting proto-eukaryote takes up a respiring bacterium to the advantage of both partners (the "Pelomyxa-stage", where lactate, the end product of fermentation, serves as the respiratory substrate for the bacterium) By the acquisition of an adenine nucleotide carrier the ATP synthesising potential of the bacterium is made available to the host cell (present-day eukaryote, containing mitochondria)

As with those features of the respiratory system, which are present in *P denitrificans* but are absent from mitochondria, so also the transport systems which are uniquely bacterial can be related to the potentially more varied and unstable environment of bacteria compared with that of mitochondria These bacterial transport systems, which would be lost in the transition to a mitochondrion, include carriers responsible for sugar transport, the periplasmic binding proteins and the extracellular iron chelators[45,46]

Both the plasma membrane of *P denitrificans* (J Burnell, P J , and F R W , in preparation) and the inner mitochondrial membrane[47] contain a sulphydryl-sensitive, phosphate carrier which mediates the uptake of P_i coupled to the simultaneous uptake of protons by an electroneutral process equivalent to the proton-symport of Mitchell[48] Thus this carrier, already present in the plasma membrane of *P denitrificans*, would require no modification to operate in the mitochondrion

On the other hand, the sulphate carriers in *P denitrificans* and in a mitochondrion seem to operate by different mechanisms In *P denitrificans* sulphate is accumulated by an electroneutral proton-symport (J Burnell, P J , and F R W , in preparation) whereas, in mitochondria, sulphate permeates the inner mitochondrial membrane principally in exchange for succinate or malate[49] Similarly, while carboxylate carriers in a number of aerobic bacteria[50-53] probably bring about the uptake of carboxylates coupled to the simultaneous uptake of protons[46], mitochondrial di- and tricarboxylate carriers act as exchange

diffusion carriers, mediating the electroneutral, equimolar P_i-dicarboxylate, dicarboxylate–tricarboxylate, and heterologous dicarboxylate–dicarboxylate exchanges[47] The carboxylate exchange carriers of the inner mitochondrial membrane clearly function to integrate the operation of the tricarboxylic acid cycle in the mitochondrial matrix with extramitochondrial metabolism[54] On the other hand, the carboxylate carriers of the bacterial membrane function simply in the accumulative uptake of carboxylates from the bacterial environment Thus in an evolutionary transition from an aerobic bacterium resembling *P denitrificans* to a mitochondrion, modification would be necessary to the mechanism of the sulphate and carboxylate carriers from a proton-symport (equivalent to an hydroxyl-exchange) to a sulphate–carboxylate, carboxylate–carboxylate, and carboxylate–P_i exchange

The only new component necessary for an evolutionary transition from the plasma membrane of *P denitrificans* to the inner mitochondrial membrane is the adenine nucleotide carrier[55], which seems to be absent from all bacteria and present in all mitochondria This carrier makes ATP, synthesised on the matrix (inner) side of the mitochondrial membrane[56], available to the ATP-utilising sites in the rest of the cell

If it be assumed that the protomitochondrion was tolerated by its host for a sufficient length of time for a stable symbiotic relationship to have developed before the acquisition of the adenine nucleotide carrier by the protomitochondrion, then it is necessary to consider what advantage this protomitochondrion may have conferred on its host for the symbiotic relationship to have survived the selective pressures to which it was exposed We suggest that the protomitochondrion, by virtue of its tricarboxylic acid cycle and respiratory chain, was able to completely oxidise fermentation products such as lactic acid and ethanol, which may have accumulated to potentially toxic levels in a relatively large and undifferentiated protoeukaryote dependent on the Emden–Meyerhof pathway for the production of ATP (Fig 3) In support of this conjecture we may note that the giant amoeba *Pelomyxa palustris*, which lacks mitochondria but contains respiratory, endosymbiotic bacteria[57-59], uses the Emden–Meyerhof pathway to supply its energy needs, produces lactic acid but has a requirement for oxygen[60], presumably for its endosymbiotic partners[58] *P denitrificans* could serve in such a role, since it can utilise both lactic acid and ethanol as sources of energy and carbon[61]

The most conspicuous morphological features of the mitochondrion are the invaginations of the inner membrane, termed cristae By contrast the plasma membrane of *P denitrificans* does not project into the interior of the cell, but remains applied to the internal surface of the cell wall[62,63] Presumably the development of cristae would be associated with the progressive specialisation of the aerobic bacterium resembling *P denitrificans* essentially into a generator of ATP by oxidative phosphorylation As this bacterium assumed its new role as a mitochondrion, the plasma membrane, as the site of oxidative phosphorylation, would increase in importance relative to the bacterial cytoplasm, which, as the site of redundant metabolic activities, would decrease in importance Reflecting these changes, we could expect an expansion in the surface area of the plasma membrane, and a contraction in the volume of the cytoplasm, thus leading to the development of cristae

Integration

By endowing the ancestor of the mitochondrion with the properties of the present-day bacterium *P denitrificans*, we have provided a plausible account of the evolutionary origin of the inner mitochondrial membrane from the bacterial

plasma membrane The major changes involved in this transition are first, the loss of redundant components of the bacterial respiratory chain and, second, a modification in the transport properties of the bacterial plasma membrane, so that effective biochemical communication is attained between the mitochondrial matrix and the rest of the cell

Many of the components of the mitochondrion that we believe to have come from the ancestral bacterium are now coded for in the nucleus and not in the mitochondrion[64,65] This implies that gene transfer has occurred during the evolution of the mitochondrion from a *Paracoccus*-like ancestor Support for this conclusion may well come from comparing the amino acid sequence of the soluble cytochrome cytochrome *c* from *P denitrificans* (when it is published) with the amino acid sequence of mitochondrial cytochrome *c*

The evidence provided here lends support to the endosymbiotic theory, firstly, by removing some of the objections raised by previous authors[40], concerning the affinity of bacterial and mitochondrial respiratory chains and, secondly, by offering a feasible evolutionary transition from a bacterial plasma membrane to the inner mitochondrial membrane This evidence on its own is, however, not incompatible with the non-symbiotic evolutionary origin of the mitochondrion proposed by Raff and Mahler[40], since their theory considers features common to bacteria and mitochondria to be "retained primitive states"[66] Furthermore, the changes which we have described for an evolutionary origin of the inner mitochondrial membrane from the plasma membrane of an aerobic bacterium resembling *P denitrificans* would also apply to an evolutionary origin of the inner mitochondrial membrane from the plasma membrane of an aerobic protoeukaryote possessing a respiratory system similar to that of *P denitrificans* Having noted these points, however, we agree with Taylor[67] that it is "unrealistic to discuss the origin of mitochondria independently from that of chloroplasts" Thus it is in the context of the greater applicability of the endosymbiotic theory to explain the evolutionary origin of the eukaryotic cell as a whole[67] that we have chosen to compare *P denitrificans* with the mitochondrion and to show how a relationship of limited advantage to both symbiotic partners could readily have developed into the present close relationship between a mitochondrion and its "host" cell

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letters to nature

Search for continuous gravitational radiation

WE have given[1] results of a search for short pulses of gravitational radiation carried out with two gravitational radiation detectors operating in coincidence over a frequency range from 650 to 1,450 Hz Detectors of that type may also be used within this frequency region to set upper limits to fluxes of gravitational radiation of either continuous or incoherent waveform The latter may include pulses which occur at a high rate but are too small to be detected individually We describe here a preliminary experiment designed to search for any such signals in a general way

If the expected signal waveform is known there are specialised methods which can optimise the sensitivity of such an experiment For example a continuous repetitive waveform of known repetition rate and wave shape, buried in the noise from a detector, can be recovered with high sensitivity by cross correlation against a reference waveform of the same characteristics,
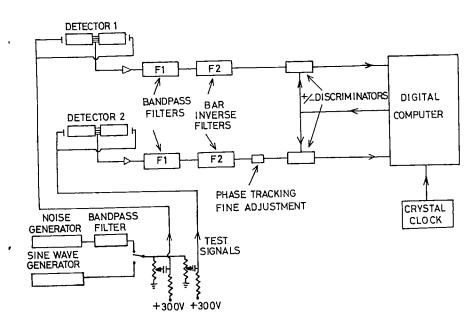
Fig. 1 Simplified block diagram of the cross correlation experiment The sine wave and noise generators shown are used for calibration F2 is a special filter with a null in its response at the resonant frequency of the bar and has the effect of giving an approximately flat non-resonant response to the whole system within the bandwidth of the bandpass filter F1

or a monochromatic continuous signal can be detected by means of a Fourier transform technique In a search for a signal of unknown type and waveform from a gravitational wave detector in the presence of noise, however, a more general technique is required The technique used in this experiment was the cross correlation of the outputs from two gravitational wave detectors This procedure allows signals common to both detectors in the presence of noise (which may contain a number of spurious detector resonances) to be recovered

Although our search was sensitive to gravitational radiation of several different waveforms, for simplicity in this preliminary experiment we tested our apparatus and set upper limits for two types of radiation first, continuous monochromatic radiation as may be produced by a rapidly rotating neutron star, and second, broadband gravitational radiation

Previous searches for gravitational waves of monochromatic and broadband type have been made at very low frequencies, although a search for monochromatic radiation from pulsars at frequencies up to 125 Hz has been reported[2] An upper limit to broadband radiation has also been set by a laser interferometer gravitational radiation detector operating above 1 kHz (ref 3) Our experiment sets a more sensitive upper limit for both broadband and monochromatic radiation in the frequency region around 1 kHz

Each of our detectors consists of two separate aluminium masses linked by piezoelectric transducers to monitor changes in separation, this arrangement giving relatively high coupling between the mechanical and electrical systems The signal from the transducers passes to a very low noise field effect transistor preamplifier and then through filters F1 and F2 (Fig 1) The overall bandwidth was set at 160 Hz, centred on 985 Hz, this being estimated as the optimum bandwidth for the detection of white noise signals This is narrower than the optimum bandwidth for a pulse search[1]

The signals from the filter F2 represent the forces acting on the detectors and are further processed and then cross correlated by a small on line computer During most of our runs the polarity of the signals from one of the preamplifiers was reversed periodically and data for reversed and normal operation were recorded separately This procedure is a useful test for any electronic coupling between the signals from the two detectors occurring at any point in the whole system after the preamplifiers The recorded data were subsequently combined taking account of the polarity reversal

Cross correlation entails the multiplication of the signal from one detector $x(t)$ by the signal from the other detector delayed by a time τ, ($y(t-\tau)$), and the cross correlation function

$R(\tau)$ for this delay is the average value of the product over the time of the experiment T

$$R(\tau) = (1/T) \int_0^T x(t)y(t-\tau)\mathrm{d}t$$

If both signals are sampled every Δt seconds for input to a computer the correlation function R_n becomes

$$R_n = (1/K') \sum_{k=1}^{k=K'} x(k\Delta t)y(k\Delta t - n\Delta t), \quad n \ll K'$$

and the variation of R_n with n can give information as to the waveform of any signal common to the two detectors In conditions of low signal-to-noise ratio, little information is lost[4,5] if the correlation is performed on polarity alone The filtered signals from the detectors are sampled and have their sign determined at regular intervals by a discriminator–latch circuit controlled through our computer by a crystal clock A '1 bit' cross correlation on the data is then computed The signs of the signals at each sampling time are recorded and a number of successive sign samples from the first detector are stored in order, this store being updated each time a new sample is taken The current sample from the second detector is compared with each of the samples from the first one and the result ($+1$ if they are of the same sign, -1 if they are different) is added to the results for previous samples In this way a number which is related to the cross correlation function is found as a function of delay between the detectors, and these numbers are displayed as our delay curves

Our maximum possible sampling rate was limited by the length of programme to 2 kHz and with this 17 correlation points 0 5 ms apart were obtained To record the delay curve with more detail in the region near zero delay, some runs were made in which the data were recorded on an analogue tape recorder and subsequently slowed down by a factor of 4 for analysis off line

Calibration was carried out by applying signals of known waveform to the capacitative endplates of both detectors simultaneously Both broadband noise and sine waveforms off the resonant frequencies of the detectors were used, the energy deposited per unit time in the detectors being measured in the first case and the force applied known in the latter case From these the amplitudes of the delay curves were found in
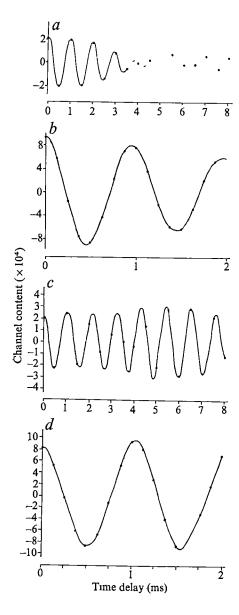
Fig. 2 Delay curves for calibration force waveforms applied to the detectors by means of capacitative endplates, *a* and *b* are the curves for an applied broadband noise waveform *a*, Obtained by on-line analysis of the data, *b*, off-line analysis of the same data recorded and slowed down by a factor of 4 *c* and *d* are equivalent curves for a sinusoidal calibration waveform at 910 Hz

number of short experiments The dotted lines in the figures indicate the root mean square deviation from the mean expected from uncorrelated data, found in this way In making a comparison with the experimental curves, allowances have to be made for the fact that successive points in any one delay curve are correlated among themselves over a time scale of about 3 ms because of the bandwidth of the system The experimental data do not show to any significant extent the waveforms in the test curves of Fig 2, do not show any persistent pattern from run to run and are in fact quite consistent with completely independent noise inputs from the two detectors The curve with the largest amplitude near zero delay is that in Fig 3*c* but the most extreme point corresponds to only 1 4 standard deviations from the mean

We conclude that the data give no indication of correlated signals from the two detectors From the measured properties of the detectors it is possible to set upper limits to fluxes of gravitational radiation signals of various types, and as mentioned earlier we can set limits on wideband gravitational flux and on monochromatic radiation in our frequency band

Fig. 3 Delay curves with no calibration forces applied to the detectors *a*, The result of an on-line analysis of a 90 h experiment (September 6–10, 1974) *b* and *c* were obtained by off-line analysis of data from two parts of a separate 24 h experiment (August 6–7, 1974), slowed down by a factor of 4 before analysis

terms of gravitational radiation flux The flux was calculated by the standard formulae[6]. Figure 2*a* and *b* shows the delay curves (measured over 52 min) for a broadband white noise signal equivalent to a gravitational wave flux of 4 3 × 10⁴ W m⁻² Hz⁻¹ averaged over the system bandwidth The shape of the curves is a characteristic decaying sinusoid the fall time of which is related to the system bandwidth of 160 Hz Figure 2*c* and *d* shows the equivalent response over 52 min for a sine wave test signal at 910 Hz, the corresponding gravitational wave flux being 2 7 × 10⁷ W m⁻²

The delay curve obtained in a run lasting 90 h with no calibration signals applied is shown in Fig 3*a*, with delays up to 8 ms Expanded delay curves obtained by analysis of recorded data from two interleaved parts of a separate 24-h run are shown in Fig 3*b* and *c* To assess the significance of the delay curves it is necessary to know the magnitude of the fluctuations which may be expected to arise purely from the uncorrelated noise in the detectors, and in the rest of the system This may be estimated, but we have also determined it empirically from independent measurements of the delay curves for a large

From the 90-h run we set an upper limit at a level of 3 standard deviations of about 2×10^3 W m^{-2} Hz^{-1} for wideband unpolarised gravitational radiation and an upper limit, again at a level of 3 standard deviations, of about 1×10^6 W m^{-2} for a continuous sinusoidal signal in the frequency range of 910–1,070 Hz, assuming the radiation is unpolarised and incident normal to our detectors for the total length of the run in each case

Our corresponding upper limit for isotropic wideband unpolarised radiation[7] is about 4×10^3 W m^{-2} Hz^{-1} and for an unpolarised monochromatic source is about 2×10^6 W m^{-2}, the exact value depending to some extent on the relative orientation of the source and the detectors

This work has some relevance to the interpretation of the pulse experiments of J Weber Observations from several groups seems to be in conflict with Weber's results but we feel that because of the differing signal processing techniques used it has not been clear that an explanation in terms of a very large flux of small gravitational radiation pulses is completely ruled out But a flux consisting of 5,000 or more pulses per day depositing an aggregate energy of about 500 times the mean thermal energy per mode in one of our detectors (500 kT), would have been observed at the three standard deviation level in the present experiment, independent of details of pulse duration and waveform If Weber's coincidences were the result of pulses corresponding to energies of 0 2 kT or less in his detectors our estimate of his experimental sensitivity, as well as the more detailed analysis of Levine and Garwin[8], suggests that the total energy flux required would be greater than the limit above

Our negative result thus provides evidence against the hypothesis that the signals reported by Weber are caused by a large flux of very small pulses

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Fig. 1 Mossbauer absorption spectrum of ^{57}Fe in thoro-steenstrupine Other spectra, spanning velocities up to 4 0 mm s^{-1} showed no evidence of any Fe^{2+} ions

bauer absorption spectrum of a 100 mg cm^{-2} sample of thoro-steenstrupine powder The spectrum is consistent with the iron appearing as Fe^{3+} in two different sites, both with octahedral symmetry[2] Most of the Fe^{3+} ions occupy a M$_2$ site with an isomer shift (relative to metallic iron) of $+0$ 40$\pm$0 03 mm s^{-1} and a quadrupole splitting of 0 34$\pm$0 03 mm s^{-1}, a very small amount (less than 5 %) of iron atoms occupying a more distorted M$_1$ site with an isomer shift of $+0$ 37$\pm$0 06 mm s^{-1} and a quadrupole splitting of 0 95$\pm$0 07 mm s^{-1} The consistency of these parameters with the systematics for other minerals[2]—which allowed the site assignments—and the relatively large intensity of the absorption lines show that the Mossbauer pattern is not affected by the radiation damage that precluded a X-ray diffraction study of the same sample, although thorium-free thoro-steenstrupine is known to crystallise with the apatite structure (J Metcalf, personal communication) This would be expected, since quadrupole splitting and isomer shift depend primarily on the immediate surroundings of the iron atom Moreover, if the crystalline environment around the metal ion were damaged appreciably, a more complex pattern with strong interactions could be expected[4]

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Mossbauer absorption in a metamict mineral

The technique and application of ^{57}Fe Mossbauer spectroscopy to minerals have been discussed fully[1-3] in the context of the determination of oxidation states, coordination numbers, and site symmetry and population This report shows, with the particular example of metamict thoro-steenstrupine {Na$_2$Ce (Mn,Fe,Ta)(La,Th,)[(Si,P)O$_4$]$_3$H$_2$}, that the Mossbauer technique can yield structural information even when X-ray diffractometry is not possible because of the destruction of the lattice ordering by the damage produced by radioactive decays

The Mossbauer spectrometer used was of conventional design, using a ^{57}Co in copper source and metallic iron calibration foils, as described previously[3] Figure 1 shows the Moss-

Mossbauer spectroscopy of Chinese glazed ceramics

For over 1,000 yr the Chinese have manufactured glazed ceramic objects renowned for their aesthetic qualities The colour of a glaze is generally achieved by the incorporation of transition metal ions[1] of which iron is the most important, being used in at least nine recognised glaze types, ranging in colour from pale blue, through green, to yellow, brown and red Although reflectance spectra of the glazes allow a simple division into types expected to contain Fe^{2+} and Fe^{3+} ions, a study of the environment of the iron in each type is of interest Such data may throw light on the technology of production

Table 1

Glaze type	Colour	Isomer shift*		Quadrupole splitting	
		Fe²⁺	Fe³⁺	Fe²⁺	Fe³⁺
Chun	Pale blue	1 08		1 04	
Celadon	Pale green	1 1	0 26	2 55	1 29
		0 82†		1 29†	
Celadon	Pale green	1 12		1 81	
		0 69†		1 38†	
Northern Celadon	Olive green		0 37		1 16
Chien (surface)‡	Rust brown		0 30		1 3
(interior)	Dark grey	1 16		2 16	
Mirror Black	Black	1 08	0 30	2 02	1 29

All measurements made at 300 K, those marked ‡ also made at 77 K Units are mm s⁻¹,
*With respect to metallic iron
†These values may be tetrahedral species
‡Also gave the spectrum of haematite

of the glazes and permit archaeological distinctions between apparently similar glazes, for example, the famous pale green Celadons

Here, I report results from a preliminary study of the Mossbauer spectra of the iron in a selection of Chinese glazes This technique has already been applied to the elucidation of the structural role of iron in experimental silicate, phosphate and borate glasses[2,3], often in conjunction with optical and electron spin resonance spectroscopic and magnetic susceptibility measurements In glasses, Fe is generally coordinated to O atoms, although traces of S or Se may coordinate to the Fe in glass, with a marked effect on the spectrum In silicate glasses the evidence suggests a fourfold or sixfold coordination for Fe³⁺ (that is, substituting for Si^{4+} in SiO_4 tetrahedra, or for such ions as Na⁺), whereas Fe²⁺ is probably always in a sixfold coordination[2] Since the ligand field and distribution of energy levels change with coordination geometry the visible spectrum is strongly influenced by the site geometry of the ions The Mossbauer spectrum is modified by the electronic charge density, by the electric field gradient, and by any magnetic field at the nucleus These are manifested as a shift in the absorption energy (isomer shift), a doublet (quadrupole) splitting, and a splitting into hyperfine structure, respectively Measurement of the isomer shift provides information on the oxidation state and coordination of the iron Values of the quadrupole splitting indicate the degree of distortion from octahedral symmetry, and moreover help to identify a particular environment, being characteristic for particular compounds Excess linewidth, beyond that found for crystalline iron compounds, gives a measure of the spread of differing environments for the Fe atoms The presence of phases with internal magnetic fields may be established from hyperfine structure in the spectrum Mossbauer spectra can decide various questions that arise in glaze studies is the range of colour from blue to green in 'reduced' glazes caused by changes in the Fe²⁺/Fe³⁺ ratio, or by changes in the Fe²⁺ environment? Is the yellow in Imperial Yellow (a lead glaze) caused by Fe³⁺ (d–d) transitions, by colloidal Fe_2O_3, or by Fe³⁺ charge transfer bands? What glazes owe their colour to Fe²⁺–Fe³⁺ charge transfer spectra? The Chien glaze (opaque rusty-brown) is believed to be caused by the formation of Fe_2O_3, how well controlled is the growth and precipitation of this phase? Is Fe_2O_3 used to produce the 'Coral Red' type of glaze? How is the 'Tea Dust' (green-brown) glaze produced?

The samples consisted of a Chun glaze (pale blue), two Sung period Celadons, a Northern Celadon (olive green), an Imperial (Ming) Yellow, an interior and surface sample from a Chien glaze, and a Mirror Black glaze Several important types are not represented, the samples were chosen by their availability for destructive analysis About 100 mg of fine powder was obtained by grinding off the glaze using a diamond wheel with the object immersed in water to prevent any atmospheric oxidation from the heat evolved by friction Mossbauer

spectra were recorded from 20–100 mg samples enclosed in thin plastic The Fe content varies from 5% (Chien glaze) to less than 1% (reduced glazes), and several days were necessary to achieve satisfactory spectra for some samples A conventional constant acceleration spectrometer was used in the transmission mode Typically, $1–2 \times 10^6$ counts were accumulated in each channel, with a maximal absorption in the range 0 5–5% Only one sample (Chien) was studied at low temperature (77 K) Most of the spectra could be satisfactorily fitted with a combination of Lorentzian curves characteristic for quadrupole doublets

The results of analysis of the spectra are presented in Table 1 and an example of the spectra obtained, together with computer-fitted component curves, is displayed in Fig 1 In general, the
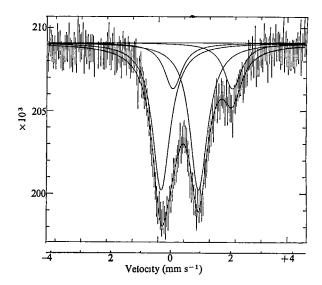
Fig. 1 Mossbauer spectrum of the Mirror Black glaze, showing its resolution into Fe²⁺ and Fe³⁺ doublets

spectra could be interpreted as sums of Fe²⁺ and Fe³⁺ quadrupole split doublets (for details, see legend to Table 1) Measured linewidths are about twice that found in crystalline compounds As is commonly found, it seems that sites giving larger splittings, within a given glaze, also have larger isomer shifts

Chun glaze. The blue colour has been attributed to the formation of vivianite, as the glaze contains phosphorus[1] The quadrupole splitting does not, however, correspond to that for the mineral, nor to that for a phosphate glass It is not evident whether the colour is caused by a modification of the characteristic 1μm Fe²⁺ band, or by a radical change in the energy level structure

Celadon glazes. Curve fitting to the weak and complex spectra is difficult, but nevertheless reveals unusual values for the isomer shift for Fe²⁺, the presence of tetrahedrally coordinated iron being indicated Despite the close visual similarity of the glazes, the two Mossbauer spectra are quite distinct

Northern Celadon. Assumed to be a more oxidised version of a Celadon glaze, this sample seems to be almost entirely Fe³⁺, with typical parameters

Chien glaze. The 300 K spectrum shows mainly a typical Fe³⁺ doublet, with a small contribution from the six-line magnetic spectrum of haematite At 77 K the spectrum is dominated by the magnetically split contribution The relaxation of a magnetically split spectrum with temperature is characteristic of a temperature-dependent spin-lattice relaxation time or of a bulk phase with large surface area Measurement of the magnetic splitting gives a magnetic field equal to that for haematite (α-Fe_2O_3) Haematite in the bulk phase does not show appreciable relaxation at 300 K, and it seems likely that the appearance of the Chien glaze is caused by the presence

of colloidal size (20–1,000 Å) particles of Fe_2O_3 (superparamagnetic behaviour) There is evidence for similar superparamagentic behaviour of haematite in clays[4] The production of such particles in a glass is of considerable intrinsic interest, and is also informative on the technical control of glaze production The interior of the Chien glaze, which is dark grey, has a spectrum characteristic of Fe^{2+}, probably as a simple doublet There may be a small contribution from magnetic phases (for example, Fe_2O_3 or Fe_3O_4) resolvable with improved signal-to-noise ratio This suggests that the Chien glaze is first developed in a reducing atmosphere, and is then superficially oxidised Such a process may be important in the production of colloidal Fe_2O_3

Mirror Black. This glaze has a very uniform reflectance spectrum in the visible region, and is produced by adding cobalt and manganese to a glaze containing 5–10% Fe The visible spectrum may be a superposition of the absorption spectra of Fe^{3+}, Co^{2+}, and Mn^{2+}, or more complex equilibria may be involved, perhaps leading to charge-transfer spectra In any case, it is interesting that the Mossbauer spectrum shows the presence of appreciable Fe^{2+} (of the order of 25%)

Imperial (Ming) Yellow. No spectrum was obtained because of the strong absorption of the lead content of the glaze

Further work on Chinese glazes must await the construction of a Mossbauer spectrometer working in the reflection mode, and capable of obtaining complex spectra from samples containing less than 1% Fe This would enable a set of representative samples to be examined without destroying the source This work is now in progress

I thank Dr A G Maddock for his help and interest, and Professor E T Hall for his encouragement Some of the sherds were kindly donated by Sir Harry Garner

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Matrix synthesis and characterisation of dichromium

MATRIX isolation has been widely used to trap reactive species in the cavities of an inert host for subsequent spectroscopic observation[1] Occasionally, spectral features appeared which could not be ascribed to the single trapped species and had to be attributed to an aggregate These bands were generally regarded as parasitic and their origin was not pursued We have discovered that metal dimers can be readily formed in large quantities in matrix conditions and that the bands associated with these entities can be identified readily using a novel technique which makes use of the fact that the metal atoms being deposited are capable of diffusion either on the matrix surface or within a narrow region (the reaction zone) near the surface before its kinetic energy is dissipated sufficiently to immobilise it

This process can be explained in terms of a kinetic model which assumes that at constant deposition rate of matrix material a steady state exists in the concentrations of the various species within the moving reaction zone Metal dimers and higher aggregates form within the reaction zone to a much greater extent than indicated by a statistical analysis based on the matrix ratio The dependence of the concentration of the various species on the rate of deposition of metal can then be
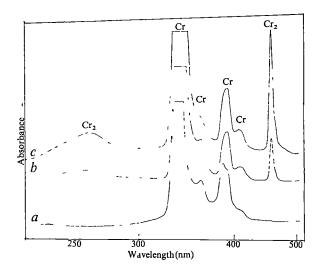
Fig. 1 A portion of the spectrum of chromium atoms and dichromium molecules in argon *a*, Low metal flow, *b* and *c*, progressively higher metal flow

calculated and compared with the observed dependence of the absorbance of bands on metal deposition rate

Using this technique we have formed and identified dimers of V, Cr, Mn, Co, Ni, and Cu and observed their spectra in the ultraviolet and visible region We use chromium to illustrate this technique

Our experimental apparatus has been described elsewhere[2] The crucial aspect of this experiment is the deposition of metal at an accurately known and constant rate A quartz crystal microbalance was used for this purpose[3] Argon (Matheson) was deposited at approximately 2 mmol h^{-1}. Chromium (McKay, New York) was deposited from a Knudsen cell lined with boron nitride at various rates ranging from a few parts per thousand to a few per cent of the argon rate.

Figure 1 shows a portion of the spectrum of chromium in argon As the rate of chromium metal deposition is increased the features at 260 and 455 nm grow Figure 1*a* was obtained by depositing Cr into argon at such a slow rate that mainly chromium atoms are found in the matrix. This spectrum is nevertheless quite different from those reported by Mann and Broida[4] and by D Gruen (unpublished) which also differ from one another Our experiences with this system suggest that the discrepancies are probably attributable to impurities and/or higher chromium aggregates in the earlier studies In Table 1

Table 1 Frequencies and assignments for matrix isolated Cr and Cr_2 in argon

$V_{Ar\ matrix}$	V_{gas} (ref 6)	$\Delta v = V_g - V_{Ar}$	Assignment	Species
29,400(vs)	$\begin{cases} 27,935 \\ 27,820 \\ 27,728 \end{cases}$	$\sim -1,600$	$a^7S \to y^7P^0$	Cr
26,900(w)	$\begin{cases} 24,971 \\ 25,010 \\ 25,089 \\ 25,206 \\ 25,359 \\ 25,548 \\ 25,771 \end{cases}$	$\sim -1,700$	$a^7S \to z^7F^0$	Cr
$\left.\begin{array}{l} 25,800(s) \\ 25,500(s) \end{array}\right\}$	$\begin{cases} 23,499 \\ 23,386 \\ 23,305 \end{cases}$	$\sim -2,200$	$a^7S \to z^7P^0$	Cr
24,300(vw)			?	Cr
38,500				Cr_2
22,000				Cr_2
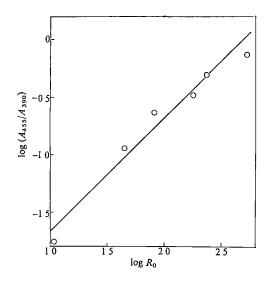
Fig 2 A log–log plot of the ratio of the absorbance of a line attributed to Cr_2 to that of a Cr resonance absorption as a function of the chromium metal deposition rate at constant argon deposition rate

we compare the frequencies of the lines measured for Cr in argon with those of the gaseous atom[5] A blue matrix shift of approximately 2,000 cm^{-1} is observed for all the lines

Unambiguous assignment of the broad band at 260 nm has not been made We know, however, that at least the band at 455 nm belongs to Cr_2 Consider the reaction

$$M \xrightarrow[k_1]{M} M_2 \xrightarrow[k_2]{M} M_3$$

which we assume takes place in the reaction zone At constant metal deposition rate R_0 a steady state exists in the concentrations of M, M_2 and M_3 given by the equations

$$dM/dt = R_0 - k_1 M^2 - k_2 M M_2 - rM = 0 \qquad (1)$$

$$dM_2/dt = \tfrac{1}{2}k_1 M^2 - k_2 M M_2 - rM_2 = 0 \qquad (2)$$

$$dM_3/dt = k_2 M M_2 - rM_3 = 0 \qquad (3)$$

where r is related to the rate at which these species are frozen out and is proportional to the argon deposition rate At low metal-deposition rates the ratio of dimer concentration to atom concentration is proportional to R_0 and the ratio of trimer concentration to atom concentration is proportional to R_0^2 (J Hulse, E P K, M M, and G A O, unpublished)

Figure 2 is a plot of log A_{455}/A_{390} as a function of log R_0 together with a line of unit slope, where A_{455} and A_{390} are respectively the absorbances of the unknown 455-nm line and the 388–392-nm Cr atom doublet The excellent correlation between the unit slope line and the experimental points suggests that the 455-nm line belongs to Cr_2 and not to a higher aggregate

The apparent absence of Cr_3 could be a result of a low extinction coefficient, or a low k_2 value, or both The latter reason may be a result of the activation energy required in the reaction

$$Cr_2 + Cr \rightarrow Cr_3$$

in which the Cr_2 bond may have to be weakened before the reaction can proceed The kinetic result, incidentally, also suggests that Cr_2 is formed in or on the matrix rather than in the gas phase This last fact is also borne out by the intriguing observation that for a given metal deposition rate the dimer–monomer ratio decreases as one increases the molecular weight

of the noble gas used to isolate them, with neon giving the most dimers and xenon the least This is understood to be a consequence of the formation of Van der Waals' complexes between the metal and the noble gas molecule with the most polarisable noble gas atom forming the strongest complexes A metal atom complexed in this fashion can either be construed to be quenched or at least its diffusion slowed down considerably

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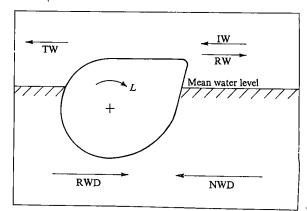
Characteristics of a rocking wave power device

THE possibility of extracting a large proportion (more than 80%) of the total wave power from water waves has been demonstrated experimentally[1] using a specially contoured rocking device In considering the possibility of large scale power generation, it is necessary to understand how such a device would respond to the variable Atlantic ocean conditions where wave periods are between 7 s and 14 s for 99% of the time[2] We here present the results of initial investigations of the bandwidth of wave periods covered by Salter's[1] device

The essential features of Salter's rocking shape (Fig 1) are a circular rear section which has a constant displacement (and therefore does not transmit any energy) and a front section with a non-uniform radius When it is matched to a wave of wavelength λ, the front section is designed to move so that it does not perturb the fluid motion of the incoming waves—which decays exponentially with depth, y, as $v_0 \exp(-2\pi y/\lambda)$—and which thus does not reflect any energy In principle, therefore, the only energy not absorbed by the device is that proportion which propagates underneath, from simple theory this would amount to $\exp(-4\pi d/\lambda)$ where d is the depth of immersion[3]

A measure of the effectiveness of the matching is provided by the difference between the body velocity and the unperturbed fluid velocity along the submerged surface, s, in the direction normal to the surface An estimate of the proportion of the

Fig 1 The Salter cam[1] TW, Transmitted wave, IW, incident wave, RW, reflected wave, NWD, normal wave direction, RWD, reversed wave direction L, load $(=T_0 K\theta)$
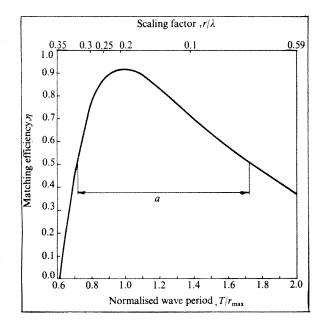
Fig. 2 Theoretical matching efficiency of a Salter cam. *a*, Half power bandwidth.

incident energy reflected or, more properly, scattered from this surface is given by

$$R = \int \overline{(v_n - u_n)^2} ds / \int \overline{u_n^2} ds \qquad (1)$$

where v_n and u_n are the body velocity and unperturbed fluid velocity (normal to the surface), respectively, and the integrations are time averaged. Equation (1) has been evaluated for the Salter shape[1], oscillating sinusoidally, and the minimum value of R has been found for a range of wavelengths in terms of a characteristic dimension of the device, r, which is taken to be the back radius. The overall absorption efficiency, η, is defined as

$$\eta = (1 - R_{min})[1 - \exp(-4\pi d/\lambda)] \qquad (2)$$

where $[1 - \exp(-4\pi d/\lambda)]$, takes account of the proportion of the incident energy transmitted underneath the device. It is plotted in Fig. 2 against the normalised dimensions r/λ and normalised wave period. Efficiencies of over 90% are predicted for $0.16 < r/\lambda < 0.20$ and the half power bandwidth exceeds 2:1.

These are most encouraging theoretical results which imply that a device 50 m in diameter, tuned to a wave of period 10 s ($\lambda \simeq 150$ m) would couple with at least 50% absorption efficiency to waves with periods of between 7 s and 17 s (75 m $< \lambda < 450$ m). We have tested these findings experimentally on a small scale in a wave tank.

The load on a device 10 cm in diameter, of the type described by Salter[1], was tuned to give maximum power output as measured by a calibrated dynamometer, from incident waves with periods, T, ranging between 0.38 and 1.0 s ($0.03 < r/\lambda < 0.22$). For periods longer than 1.0 s, assumptions of deep water conditions ceased to apply in our tank. Waves with periods of less than 0.38 s were attenuated significantly. Over the intermediate range, attenuation could be ignored and then the incident and reflected wave amplitudes, A and B, respectively, were calculated from the standing wave ratio, S, defined by:

$$S = (A - B)/(A + B)$$

where $(A - B)$ and $(A + B)$ are, respectively, the minimum and maximum amplitudes measured on the standing wave in front of the device. The amplitude, C, of the wave transmitted past the

device was also measured. The incident, reflected and transmitted powers were calculated (see ref. 3) from these amplitudes respectively:

$$(P_i, P_r, P_t) = (\rho g^2 T/8\pi)(A^2, B^2, C^2)$$

and the proportion of power lost from the wave, W, is

$$W = [P_i - (P_r + P_t)]/P_i$$
$$= 1 - (B^2 + C^2)/A^2$$

W is plotted against T in Fig. 3*a*. This curve is very similar to the predicted efficiency curve (Fig. 2). We also obtained a direct
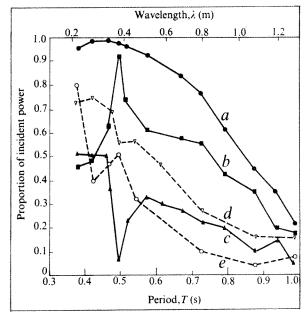
Fig. 3 Efficiency and losses for the Salter cam. *a*, W (tuned); *b*, η_g; *c*, losses (tuned); *d*, W (free); *e*, W (fixed).

measurement of the power absorbed by the device at the dynamometer, and this is plotted (Fig. 3*b*) normalised to the incident power P_i, and corresponds to the actual efficiency of power extraction η_g. Though both curves peak at over 90%, the useful power output curve suggests that the device/load combination is resonant at $T = 0.5$ s ($r/\lambda = 0.13$) with a rapid fall off in efficiency to about 60% on either side. The difference between Fig. 3*a* and 3*b* represents the proportion of the incident power lost from the wave system because of turbulent and other nonlinear effects (Fig. 3*c*). Although small at the 'resonant' period (about 5%) these losses are, in general, surprisingly large, as high as 50% for the shorter periods.

Losses will be very important in practical systems and so they warrant further investigation. To assess the importance of the motion of the device in causing these losses, the experiments were repeated with the device either oscillating freely or held rigid (Fig. 3*a* and *b*). As no power can be extracted by the device in these cases, W can only represent system losses. Over most of the period range the losses measured for optimally loaded system lie between those for the two extreme cases. This is not an unexpected result and suggests that the system losses increase monotonically as the amplitude of motion of the device increases. At the 'resonant' period, however, the matched device losses are only about one-tenth of the fixed and free device losses which are both about 50%, which suggests that velocity mismatch in the normal direction contributes more to losses than do shear velocity gradients.

To see whether power was being lost or propagated in some preferred direction of rotation, the reciprocity of the system[4]

was measured by repeating our measurements with waves incident in the reverse direction, that is, on the rear of the device. We found that the same fraction of power $(C/A)^2$ was transmitted in either direction within experimental error over the whole range of wavelengths, and so the system was reciprocal.

For those losses caused by boundary layer effects and turbulence, the fractional power loss will be dependent on the Reynold's number (Re). As λ is proportional to T^2 (ref. 3), Re is proportional to T^3 and the Reynold's numbers at full scale will be nearly 10,000 times greater than in our experiments, and these losses would be greatly reduced.

Experiments with our model have shown that good power conversion efficiencies (more than 50%) can be obtained over the range of wave periods (a 2:1 bandwidth) corresponding to that found in ocean waves. Further work is needed to establish in detail the response to complex wave fields with multiple frequencies from various directions, the effects of real sea conditions, the effect on performance of alternative loading systems and the precise way in which losses scale. It is, however, already clear that the rocking boom device is very promising as the basis of a wave power system. The practical effects that we have investigated, of losses and bandwidths, do not seem to represent serious limitations on performance.

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Relation of abnormal earlywood in oaks to dendrochronology and climatology

TREE-RING investigations in Europe are largely based on the method initiated by Huber in 1938 at Tharandt (near Dresden)[1] by which the widths of the rings are measured and the resulting sequence charted and compared both visually and by computer. In temperate zones such as England and the Low Countries oak panels are ideal for the study of dendrochronology as they were prepared from the butt logs of elite trees of slow and even growth. A panel used as a support for a painting has the added advantage that its edges are accessible[2]. Since 1971 I have worked on 115 of those used by portrait painters in South-east England or Flanders between 1450 and 1620 (refs 3, 4). The boards (there are more than one on some panels) have an average of 200 rings of mean width 1.5 mm.

At an early stage a curious feature was noticed on the panel of Holbein's portrait of Robert Cheseman; all the vessels in the earlywood of two of the annual rings are abnormally small (Fig. 1). This peculiarity has subsequently been observed in about 0.4% of the 24,000 rings measured on these panels and often, but not invariably, appears in the same annual ring on both ends of a board as well as on separate boards prepared from the same tree. I have also found examples on the boards of a late mediaeval cupboard (Fig. 1) made for an Oxford College[5], and on timbers used for the Roman quay to the Thames at London, but none in the stems of 30 trees[5] felled recently at an age of about 150 yr.
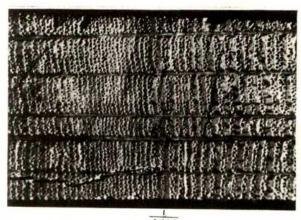
For the panels (almost all their ring-width charts have been matched and dated by reference curves) it soon became apparent that the effect was mainly confined to three specific years in the period 1433–55. At present this period is covered by 109 dated boards derived from 81 trees. Examples occur on 47 of these boards (derived from 36 trees) there being 36 occasions for the year 1437; 12 for 1443; and 21 for 1454. Six of the same boards also had earlywood vessels smaller than usual for one or more of the years 1438, 1439, 1444, 1445, and 1455, the total of such instances being ten. In the fourteenth century the abnormality appears for 1348 and 1390 (with eight and five occasions respectively); the remaining 20 examples are distributed sporadically between 1260 and 1540.

The presence of earlywood vessels has proved to be diagnostic for dating. They were observed in two rings separated by 17 yr on the portrait of Elizabeth Woodville in the Royal Collection at Windsor Castle. With the dates 1437 and 1454 thus suggested for the two rings, the 116-yr sequence matched well with signatures on one of our reference curves (Fig. 2). It is therefore dated with high reliability even though it is relatively short in length because of an imperfection on part of the edge.

Vessel development commences almost simultaneously throughout the stem cambium of a large oak by very complex processes[6]. When the buds flush in April–May, the vessels expand apparently under the stimulus of auxin and other compounds. K. A. Longman and K. R. Shephard have suggested (personal communication) that interference with cambial activity by a climatic factor, perhaps limited to only a few days and directly or indirectly related to flushing, might cause earlywood vessels to be abnormally small. A purely local cause can be dismissed as examples occurred in 1433 in woodlands near Oxford, near London and in the Low Countries. The effect in the period 1433–55 may

Fig. 1 Part of the edge, 1.8 cm wide, of an oak board, with 277 annual rings spanning 1209–1485, showing abnormally small earlywood vessels for 1437. This board and another form one of the 18 doors of a large cupboard made for Corpus Christi College, Oxford, in the period 1515–20, when the College was newly founded.

1437

Direction of growth ⟶

We have also attempted to study the dependence of the photovoltaic effects of the Chl-*a* sandwich cells on the electrode materials[5]. A correlation between the photovoltaic activity and the work function of the metals is observed, which further indicates that the Schottky barrier is directly responsible for the photoactivity. For instance, in a (Cr|Chl-*a*|Ga) cell, where Ga has a lower work function than Cr, the dominant Schottky barrier is established at the back, Chl-*a*|Ga, contact. The observed photovoltaic activity is dramatically different. The sign of the i_{sc} is now in a direction opposite to that seen in the (Cr|Chl-*a*|Hg) cell. Furthermore, the i_{sc} spectral response shows maxima in the spectral regions where the Chl-*a* film only absorbs slightly, that is, an "inner filter" effect is observed.

Table 1 Photovoltaic characteristics of ten (Cr|Chl-*a*|Hg) cells

Cell	V_{oc} (V)	i_{sc} (in 10^{-8} A)	P_{max} (in 10^{-8} W)	P_{max}/P_{in} (in 10^{-2} %)
1	0.32	8.0	1.00	1.6
2	0.31	6.0	1.08	1.0
3	0.18	3.9	0.43	0.43
4	0.19	7.3	0.47	0.68
5	0.18	5.0	0.30	0.50
6	0.21	5.0	0.38	0.63
7	0.18	8.0	0.48	0.80
8	0.30	7.5	0.80	1.40
9	0.20	6.0	0.50	0.83
10	0.25	2.5	0.40	0.67

V_{oc} is the open circuit voltage, i_{sc} is the short circuit current at the 745 nm light intensity of 7.6×10^{-4} W cm^{-2}. P_{in} is the incident radiative power on the cell (no correction is made for the power absorbed by the Cr). P_{max}/P_{in} is the photovoltaic power conversion ratio.

The Schottky barrier model for the system predicts an activition energy for a forward bias current which consists of a voltage dependent term, $-eV/n$, and the barrier height (contained exponentially in i_0) at the Cr|Chl-*a* contact[8]. Accordingly, after correcting the observed activation energy for the forward bias voltage, barrier heights on the order of 0.4–0.7 eV are obtained, depending on the sample. Measurements of activation energies on a single sample at different bias voltages, or with cells having different electrode materials should test further the contact barrier model for the Chl-*a* system. The activation energy seen for the photovoltaic current seems to be exceptionally low for organic systems. Evidently, the steps leading to charge generation and transport following light absorption by Chl-*a* involve at most very weakly activated processes.

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Can linguistic competence be dissociated from natural language functions?

The preservation of non-verbal intellective functions in spite of severe disruption of natural language has been well documented by studies of aphasia[1]. But are the cognitive functions specific to normal verbal communication also preserved in the absence of language? In addition to its theoretical significance, this question has practical importance with respect to both infrahuman primates and severe aphasics[2]. These groups cannot use natural language because of the destruction or apparent absence of crucial cortical zones of the brain and effective communication may depend on their mastering an alternative symbol system which captures relevant cognitive properties of natural language. A crucial test of the hypothesised dissociation between natural language and its cognitive prerequisites is whether aphasic subjects can use arbitrarily designed symbols to represent elements of experience (events, properties, actions and so on); encode meaningful relationships in terms of the configurational properties, or syntax, of the symbol sequence; and have the capacity to encode such relationships in alternative syntactic forms[3,4].

We have verified the preservation of these cognitive concomitants of natural language in a severely aphasic patient. W.S. is a 21-yr-old right-handed male who was evaluated for language after surgical removal of a large, acute subdural haematoma of the left tempero-parietal lobe following severe head trauma. Ten weeks after surgery, at the time of admission to our unit, he had mild weakness in his right seventh cranial nerve and probable loss of smell sensation on the left; the remainder of his neurological and physical examination was normal. At this time he was observed to have very poor comprehension of spoken and written language. His speech was markedly paraphasic and devoid of information. On the Boston Diagnostic Aphasia Examination[5], he scored near zero on subtests of auditory comprehension, naming, reading comprehension and writing. But he seemed to be extremely well oriented and alert, and when his intelligence was tested he executed the block design, picture arrangements and object assembly subtests of the Wechsler Adult Intelligence Scale at a superior level (scale scores of 12).

W.S. entered our research–therapy programme called VIC (Visual Communication) in which messages are formed by laying out—from left to right—index cards on which arbitrary or ideographic symbols stand for nouns and verbs, grammatical morphemes, sentence modality and tense markers, and metalinguistic signals. The patient observes VIC communicators 'converse' with one another and then is gradually drawn into the conversation himself. Within two 30-min sessions W.S. had mastered the basics of the system; he demonstrated knowledge of the meaning of VIC symbols and the rules of the syntax governing VIC communication. Specifically, he could follow commands, answer simple questons, and describe events, with the total number of possible transactions running into the hundreds. After 14 additional 30-min sessions of VIC, W.S. could transmit and receive information involving direct and indirect objects, antonymic contrasts of verbs and of prepositions, judgments on the truth value of statements, and shifts in sentence modality, for example, from the interrogative to the declarative. More practically, W.S. was able to use the cards to express certain feelings and ideas; clearly VIC served as an alternative for his vitiated natural language capacity.

Here is a sample annotated exchange, translated into English. Word sequences designated by a single ideograph are connected by hyphens.

(1) Command involving a reordering of the element in the surface form of the utterance: Therapist (T): ! W.S. please-put spoon or fork to-the-right-of-the pencil. W.S. executes act with fork.

(2) Answering question requiring multiple specification of nouns and expansion of constituent: T gives pencil and razor to Y. T: ? Who give(s)-to what (to) whom. W.S.: T give(s)-to pencil and razor (to) Y.

(3) Appropriate response when confronted with a novel surface form: T picks up a cookie. T: ? What T pick-up [rather than normal form: ? T pick-up what]. W.S.: T pick(s) up cookie.

(4) Spontaneous utterance in VIC: T takes out pack of cigarettes. W.S.: W.S. want(s) cigarette (a few seconds elapse) and matches.

To establish that this success was not the result of a mapping of VIC on to residual or recovering natural language functions, W.S. was given an extensive test of spoken and written English, with all items equivalent to VIC messages. He performed without error on items in VIC, but scored only 12.5% correct in English.

The design of VIC suffices to demonstrate important conceptual operations entailed in ordinary language. First, W.S. seems to understand and use the symbols in terms of the normal categories of perceptual experience (for example, actor- action- object and indirect object of the action). Second, W.S. seems to understand the meaning relations among the categories in terms of a small number of configurations and to deal appropriately with novel mesages; both of these capacities suggest that he can understand VIC sequences in terms of the relations among the component elements. Third, W.S. is not bound by referentially constrained situations in his use of VIC. Finally, W.S. seems to exhibit the beginnings of productive use of VIC, by virtue of his ability to expand on constituents that request information.

The case of W.S. shows that certain cognitive capacities entailed in natural language can be preserved in its absence. This patient was unique among VIC communicators in the speed and facility with which he mastered the system. The fact that five out of eight patients tested have mastered the basic components of the system within 6 weeks, however, and have performed at a level superior to their performance with English, indicates at least some dissociation among the two functions in severely aphasic patients.

The performance of W.S. can be compared usefully with those of Sarah[6], Washoe[7] and Lana[8], primates used in communication studies; however, his achievement differs not only in its overall level of proficiency, but also in the apparent fact that Sarah has not used her symbol system for spontaneous communication while Washoe has not used syntax consistently. The skill of W.S. at mapping his knowledge of the environment on to a symbol system without the apparent aid of a mediating system is most reminiscent of that found in young children, and, perhaps, in Lana. Possibly our patient is in the paradoxical position of retaining cortical capacity for communication using symbols while having lost, at least temporarily, the ability to handle natural language.

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Intensification of the alcohol withdrawal syndrome by repeated brain stimulation

SINCE the discovery that electrical stimulation of discrete sites of the brain can influence behaviour, there have been numerous reports of its application[1]. Such procedures, however, may lead to kindling[2]; stimulation of some sites at currents initially too low to produce a behavioural response may lead to a gradual development and then intensification of seizures evoked by the stimulation[3]. Thus when the brain is repeatedly stimulated for therapeutic purposes, seizures may be elicited unless procedures not conducive to kindling are used[2]. Two questions arise: can kindling leave an organism more susceptible to seizures produced by agents other than local brain stimulation, and are elicited convulsions necessary for this change in susceptibility? Our findings indicate that whether or not overt convulsions are produced by brain stimulation, repeated electrical stimulation of the brain potentiates the effects of subsequent alcohol withdrawal.

To answer the first question we studied the interaction of kindling and alcohol. When large quantities of ethanol have been metabolised an organism is in a state of heightened seizure susceptibility[4]. In alcoholics this withdrawal syndrome is often characterised by tremors and other mild epileptic symptoms, and *grand mal* convulsions and death can ensue. Even in organisms that display no obvious symptoms, there is an increased susceptibility to seizures induced by other agents after withdrawal[4]. Hence, if kindling affects general seizure susceptibility, this should be reflected in an intensification of the alcohol withdrawal syndrome.

Twenty-seven, male, black-hooded rats (Canadian Breeding Farms, La Prairie, Quebec) were implanted with unilateral, bipolar electrodes aimed at the centre of the amygdaloid complex. After 1 week of recovery, 12 randomly selected rats were kindled with 45 brain stimulations (1 s, 400 µA, 60 Hz) administered three times per day, 5 d per week. The animals were kindled successfully in that each eventually displayed at least one bilateral seizure involving clonic activity of the head, mouth and fore-limbs accompanied by rearing and loss of equilibrium. The remaining implanted animals served as unstimulated controls.

Three days after the last stimulation, rats in both groups received 45 intubations of a 20% volumetrically prepared alcohol solution (1,000 mg kg^{-1}) as before[5]. Intubations were at 8-h intervals and intoxication was tested 1 h after each intubation. An animal was considered intoxicated when it exhibited two or more of the following symptoms: (1) unconsciousness before handling, (2) inability to manoeuvre on a vertical screen or (3) ataxia. If there were no signs of intoxication, subsequent doses were increased by 200 mg kg^{-1} until intoxication was produced. All animals developed a tolerance to the alcohol; the mean difference between the first and last intoxicating dose was 870 mg kg^{-1} and every animal showed an increase of at least 200 mg kg^{-1} ($\chi^2 = 20.02$; $P < 0.001$). Before withdrawal the body weights (mean = 342 g) and effective intoxicating doses (mean = 2,018 mg kg^{-1}) were similar for stimulated and unstimulated animals.

Commencing 9 h after the last intubation each animal was placed under formal observation for 2 min every 3 h by an experimenter, unaware of the animal's experimental history, who recorded the presence or absence of the following seven withdrawal symptoms: rhythmic activity of the mouth, facial tremors, rhythmic eye or ear twitching, myoclonic body jerks, tail tonus, rhythmic head nodding and hyperreactivity to handling. Since there were six observation sessions, the maxi-
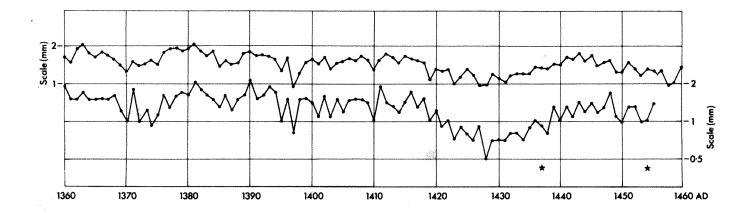
Fig. 2 A 100-yr section of the ring-width chart (semi-log scale) for the oak panel support of the portrait of Elizabeth Woodville It agrees closely with the signatures on the reference curve. The stars mark the two years with abnormally small earlywood vessel. Upper, Reference curve (18 trees, left hand scale); lower, Elizabeth Woodville (right hand scale).

well be linked with the exceptionally severe winters and cold springs[7-9] in central and western Europe between 1428 and 1450, but more specific factors must have been involved as it did not coincide with each. Whatever the cause, there is no indication that the width of the subsequent latewood was significantly altered.

Both Flohn[10] and Lamb[8,9] associate the cold winters and springs of the 1430s and 1440s with a long period of blocking conditions which lessened the frequency of the westerly winds over Britain and much of north-western Europe. Since the annual rings of numerous oaks that have remained buried from about 6000 BC are now being measured at Stuttgart[11], Cologne[12] and Belfast[13], observations of abnormal earlywood may extend our knowledge of variations in Europe's climate in Flandrian times.

This research has been supported by the Leverhulme Trust Fund. It owes much to the permission graciously granted by Her Majesty The Queen to work on paintings at Windsor Castle, and to the co-operation of the Surveyor of the Queen's Pictures, the Director of the National Portrait Gallery, the Society of Antiquaries and owners of private collections. I thank the members of the Department of Forestry for help with the computer work.

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Chlorophyll-*a* photovoltaic cells

ALTHOUGH the properties of an organic photovoltaic cell in which the photoactive material is microcrystalline chlorophyll-*a* (Chl-*a*). In a sandwich configuration having chromium and mercury as the electrodes, the Chl-*a* cell, (Cr|Chl-*a*|Hg), achieves a power conversion efficiency of the order of 10^{-2}%, which is among the highest ever reported for organic photovoltaic cells[1,2]. In contrast to the usual methods for preparing thin films (such as vapour deposition), a thin microcrystalline Chl-*a* film is prepared on a Cr electrode surface by he simple method of electrodeposition[3]. Such a film is fairl; uniform and is uniquely efficient in the photocharge generation. The microcrystalline form of Chl-*a* differs from the aggregated form of monomer Chl-*a* in that it has an ordered structure and has a strong absorption band in the far red, peaking at 740-745 nm. A water-Chl-*a* adduct structure has been proposed for this type of Chl-*a* (ref. 4).

The fabrication of the metal-Chl-*a*-metal sandvich cells (including the (Cr|Chl-*a*|Hg) cell has been descrbed elsewhere[5]. The electrodeposited Chl-*a* films used in most of the Chl-*a* photovoltaic cells are about 1,500 Å thick as measured by interferometry. The corresponding optical density of the Chl-*a* film at 745 nm is about 1.5. Monochromatic ilumination is through the semitransparent Cr electrode (~ 30% transmission). The intensity of the light incident on the Cr electrode is about 10^{-3} W cm^{-2} at 745 nm.

The electrical conduction of the (Cr|Chl-*a*|Hg) cell in the dark exhibits a strongly non-ohmic or rectifying behaviour. The cell is found to be forward biased when a negaive voltage is applied to the Cr electrode. Figure 1 shows a semiogarithmic plot of the dark current against applied voltage, the $-V$ characteristics, of a (Cr|Chl-*a*|Hg) cell. The rectification ratio (forward bias current/reverse bias current) is as high as 10^3. The photoconduction behaviour is shown in Fig. 2. Curve (*a*) represents the same dark *i*-*V* characteristics as in Fig. 1 but plotted on a linear scale. Curves (*b*) and (*c*) represent the photocurrent as a function of applied voltage photo *i*-*V* characteristics). These two curves are obtained when the cell is illuminated by 745 nm light having intensities o 7.6×10^{-4} and 2.7×10^{-3} W cm^{-2} respectively. The insert in Fig. 2 represents the photovoltaic *i*-*V* characteristics. Hee there is no applied voltage. Instead, photovoltage alone is present and
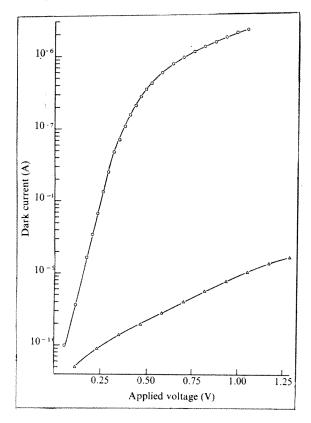
the cell current is varied by changing the external load resistance, R_L in series with the cell from 10^4 to $10^{11}\Omega$. In this case the cell is under constant illumination by 745 nm light at a level of $.6 \times 10^{-4}$ W cm^{-2}. Note how the shape of this purely photovoltaic i-V curve is very similar to the lower right quadrant portion of the i-V curve (1). The area of the largest rectangle enclosed by such an i-V curve represents the maximum power obtained from the cell at this light level. This maximum power of 1×10^{-8} W is found to couple through a R_L of $3 \times 10^6\Omega$. With an incident power of 6×10^{-5} W at 745 nm on the Hg contact area (0.08 cm^{-2}), the power conversion efficiency is about $1.6 \times 10^{-2}\%$. If correction for the light absorbed by the Cr layer is made, the efficiency is more nearly $5 \times 0^{-2}\%$. The open circuit voltage, V_{oc}, is about 0.32 V for this cell, and is nearly independent of the light intensity at this light level. On the other hand, the short circuit current, i_{sc} shows a linear dependence on the incident light. The ratio i_{sc}/I, where I is the photon flux upon the Chl-a (that is, corrected for absorption by Cr) is 0.007. This represents the minimum quantum yield of charge generation in Chl-a when the cell is operating in the photovoltaic mode.

It must be noted the cell described above is one of the best found in this study. The performance can vary by as much as an order of magnitude from the poor to good cells. Table 1 summarises the data obtained from ten (Cr|Chl-a|Hg) cells. The average power conversion efficiency is about $5 \times 10^{-3}\%$. These cells are very stable and retain their characteristics for at least a month (in the dark) without obvious deterioration. Slow degradation under more intense light ($> 10^{-2}$ W cm^{-2} at 745 nm) is seen. At the light level of 10^{-3} W cm^{-2}, a good cell can sustain a constant i_{sc} of the order of 0.1 µA over a period of hours.

Temperature dependence studies have also been performed on some of the cells. Between room temperature and ~ -40 °C the photovoltaic i_{sc} exhibits a very small dependence on

Fig. 2 i-V characteristics. (a), Cell unilluminated; (b) and (c), cell illuminated by 745 nm light of intensities 7.6×10^{-4} and 2.7×10^{-3} W cm^{-2} respectively. The insert is the photo i-V characteristics of the (Cr|Chl-a|Hg) obtained by varying the load resistance while the cell is constantly illuminated by 745 nm light.
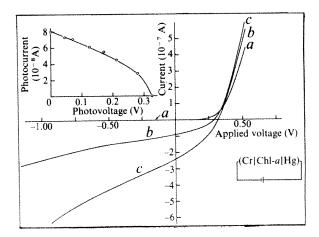
temperature, whereas the dependence of the dark forward-bias current is considerably stronger. The Arrhenius plots give an activation energy of less than 0.05 eV for the photovoltaic i_{sc} and for the dark forward bias current values of 0.2 to 0.5 eV are found, depending on the sample.

The remarkable properties of the (Cr|Chl-a|Hg) photovoltaic cell may be explained in terms of the existence of a contact potential barrier (or Schottky barrier) at the Cr|Chl-a contact. Chl-a has been shown to exhibit p-type semiconduction[6]. It is generally true that a potential barrier often exists at the contact between a p-type semiconductor and a metal of low work function[7]. Thus, in the (Cr|Chl-a|Hg) cell the dominant Schottky barrier is at the Cr|Chl-a boundary, while the Chl-a|Hg contact possibly can be ohmic, or only slightly blocking. The asymmetric potential distribution provides the basis for the dark current rectification and the photovoltaic characteristics. The forward bias dark current in Fig. 1 is seen to follow a modified Shockley equation[8] for a rectifying contact: $i = i_0[\exp((eV - iR_s)/nkT) - 1]$ where $i_0 = 2 \times 10^{-11}$ A and $n = 1.6$. The iR_s term represents the potential drop across the series resistance, R_s, in the (Cr|Chl-a|Hg) photovoltaic cell. Its value is found to be $1.2 \times 10^5\Omega$ and is orders of magnitude larger than that found in inorganic photovoltaic cells such as the silicon solar cell[9].

The action spectrum of i_{sc} of the (Cr|Chl-a|Hg) cell is seen to match closely the absorption spectrum of the Chl-a film[5]. The optical thickness of the Chl-a film at 745 nm is such that were the photoactive domain located at the back contact, Chl-a|Hg, a significant "inner filter" effect (a quenching of the photoelectric response) would be seen. More detailed analysis[5] has shown that the width of the photoactive region in the Chl-a film adjacent to the Cr electrode is a few hundred angstroms. Light absorbed by the bulk of the Chl-a beyond this region is presumably not very effective in producing free carriers. The minimum quantum yield of charge generation in the Chl-a film which is 0.007 with no bias, is found to increase under reverse bias (see lower left quadrant of Fig. 2). The reverse bias, which increases the barrier dimensions, evidently serves to partially overcome otherwise inefficient steps involving primary charge generation or subsequent recombination events, or both[10]. Meilanov et al.[6] have studied (|Al|Chl-a (non-crystalline) Al) cells at thicknesses far exceeding ours and at much higher applied voltage. Studies in the photovoltaic mode were not presented. A voltage dependent yield as high as 0.1 was observed however.

Fig. 1 Dark i-V characteristics of a (Cr/Chl-a|Hg) cell. Note the forward and the reverse biased currents have opposite signs. ○, Forward bias; △, reverse bias.

mum score an animal could receive for each symptom was 6, with an overall maximum of 42 for all symptoms combined.

The withdrawal syndrome observed in kindled animals was much more severe than that of the controls in terms of each symptom. To make an overall statistical comparison each animal was given a score for each symptom that was a percentage of the mean score obtained for the unstimulated control animals for that symptom. Thus, the mean of the control animals for each symptom was 100% and the control mean for all seven symptoms was also 100%. In contrast, the mean of these overall scores for the kindled animals was 249.8% ($U = 16$; $N_1/N_2 = 11/110$ $P < 0.005$, one-tailed). The incidence of seizures characterised by rhythmic head nodding, synchronous jaw movements and facial spasms over the six observation periods was also significantly greater in kindled animals (mean = 3.5) than controls (mean = 1.2) ($U = 18.5$; $N_1/N_2 = 11/11$; $P < 0.005$, one-tailed).

Repeated brain stimulation not only leads to seizures but also to a permanent decline of the afterdischarge (AD) threshold[6]. Kindling occurs only when the stimulation levels are above the AD threshold, but this threshold can be reduced even when stimulation has no electrographic or behavioural effects[6]. Thus even stimulation with no obvious effects can produce a lasting change in brain function.

To investigate whether such stimulation can intensify subsequent alcohol withdrawal, or whether elicited convulsions are necessary 11 animals were implanted with electrodes as before and an initial AD threshold was determined for each. Each animal was stimulated for 1 s with 25 µA, then, if no AD was elicited, with 50, then 75 and so on at increments of 25 µA every 6 h until an AD was produced. The intensity that evoked an AD was considered the threshold for that animal. Each animal was stimulated until only one AD was produced and then handled without stimulation until all thresholds had been determined. Subjects were then divided into two groups on the basis of their thresholds. One group ($n = 6$; mean = 131.2 µA) was placed on a stimulation regimen similar to the schedule of the first experiment, with an initial stimulation intensity for each animal 15% below the threshold for that animal. Whenever an AD was produced, the intensity of subsequent stimulations was reduced by another 15%. In this way most stimulations were kept below the AD threshold. Thus, although the average threshold drop for the stimulated groups was 62.2%, no animals developed full motor seizures, and only one displayed mild behavioural symptoms in response to stimulation. The remaining animals ($n = 5$; mean = 134.3 µA), served as unstimulated controls. Two days after the last stimulation all animals received alcohol intubations and underwent withdrawal as in the first experiment.

The raw scores for each withdrawal symptom were again converted to a percentage of the control mean for respective symptoms. As in the first experiment each symptom was more frequent in stimulated animals than in controls and the overall means (100.0% compared with 197.1%) differed significantly ($U = 3$; $N_1/N_2 = 5/6$; $P < 0.02$).

Our data show that repeated brain stimulation not only increases susceptibility to seizures produced by subsequent stimulations, but also renders the organisms more susceptible to other convulsive agents. Intensification of withdrawal symptoms by stimulation above or below the AD threshold suggests that the effect does not depend exclusively on the production of overt seizures, and that the reduction of the threshold increases the severity of withdrawal.

If brain stimulations are repeated for several months, spontaneous seizures occur, even after all stimulation ends[7,8]. Thus, we think kindling reflects the development of an epileptic focus. Since the effects of various convulsive agents interact with epileptic foci, alcohol is probably not the only agent whose withdrawal symptoms are potentiated by prior kindling. More importantly, as ADs have been produced repeatedly in humans[9,10] and as clinical evidence exists of kindling-like phenomena[10], it may be necessary to re-evaluate some clinical practices, with regard to both possible kindling in brain stimulation treatments,

and the subsequent administration of convulsive drugs. The important consideration in such a re-evaluation is that even when the current intensities are too low to produce any electrographic or behavioural effects, enduring changes in brain function may be produced.

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Aggression and mating success in *Drosophila melanogaster*

THE value of *Drosophila melanogaster* as an experimental animal for the study of the genetics of behaviour increases as more specific behaviours are shown to exist in this species. Although aggressive behaviour has been reported for *D. subobscura*[1] and some Hawaiian[2] species, and has been briefly mentioned for many other species[3], it has not yet been described for *D. melanogaster*. We have repeatedly observed behaviours which we interpreted as aggressive and here, report an experiment designed to investigate aggression and its relationship to mating success—an important component of fitness.

Flies from a mass-bred stock (Haren) which has been in the laboratory since July, 1971 were used in this experiment. To identify individuals, 12-h-old males were marked on the dorsal surface of the thorax with dots of acrylic paint while under light etherisation. They were kept singly in vials containing standard *Drosophila* medium until they were 3 d old. At this time, six males were placed in a cylindrical Perspex arena (7 cm diameter, 4 cm high) containing a small dish (2.5 cm diameter) with *Drosophila* medium. After an 18-h settling period, flies were observed with a binocular microscope. All interactions occurring on the food surface and their outcomes were recorded over a period of 3 h. In addition the

Fig. 1 A charging male of *D. melanogaster:* wing threat, wings spread, raised and twisted.
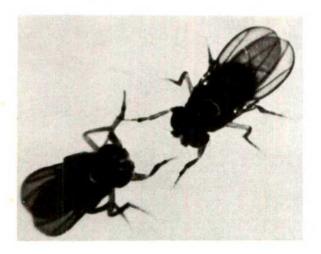
Fig. 2 Two males boxing.

position of the individuals in relation to the food surface was was noted every 5 min. We then started to introduce 3-d-old virgin females at intervals of approximately 50 min, recording which males were successful in copulation. At the end of each day the females were removed.

The interactions observed were primarily aggressive or sexual. We found three frequently occurring aggressive behaviour patterns: wing threat (Fig. 1) which is often directed to other males just before a very quick charge, in which the aggressor usually rises on his hindlegs shortly before impact is made, and boxing (Fig. 2) which comprises several variations of very vigorous slashing and tapping with the front legs, often while both males rise on their hindlegs.
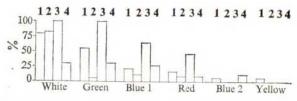
Fig. 3 Activities of each male, expressed as percentages. (1) Time spent on the food, 100% = total time of observation; (2) attacks initiated, 100% = total number of attacks made by all flies; (3) fights won, 100% = total of a given animal's fights; (4) copulations, 100% = total number of copulations by all males = 19.

The six males showed large differences in the frequency with which they stayed on the food surface, with which they attacked, were attacked, won and lost bouts of aggression and copulated (Fig. 3). The winner is defined as the animal which remains on the food surface after an aggressive encounter. Clearly, 'white' stayed on the food surface most often ($\chi^2 = 64.1$, 5 d.f., $P < 0.005$), initiated most threats and attacks relative to the amount of time spent on the food ($\chi^2 = 31.0$, 3 d.f., $P < 0.005$) and won 100% of his fights. Ranking the animals on the proportion of fights won gives the following result: 'white' = 'green' > 'blue 1' > 'red' > 'blue 2' = 'yellow'. This rank order is significantly correlated ($r = 0.95$, $P < 0.01$) with the number of copulations achieved by each male.

Drosophila males do court other males, and other investigators who have encountered the behaviours we have described may have interpreted them as sexual in nature. In well over 1,000 videotaped single pair courtships, however, none of the behaviour patterns that we define as aggressive has previously been observed. Milani[1] has described a behaviour

similar to our wing threat in *D. subobscura* and interpreted it as aggressive in nature. Brown[4] dismisses this notion and prefers to call it counter-signalling. According to our observations, however, it cannot be a counter signal since a male engages in this behaviour before an interaction initiated by him. In *D. melanogaster*, wing flicking serves as a counter signal which is given in interactions involving physical contact between individuals of either sex.

The adaptive nature of aggression on a food source might be that males monopolise a food resource for reasons of energy requirement and/or because females will be attracted to it. Our results provide evidence that the more successful a male is in fights the fitter he is in terms of mating success.

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The function of phytochrome in plants growing in the natural environment

MANY aspects of plant development are subject to photocontrol by way of the chromoprotein photoreceptor phytochrome. Phytochrome exists in two forms, P_r, which absorbs maximally at 660 nm, and P_{fr}, which absorbs maximally at 725–735 nm[1,2]. Absorption of light by either form results in phototransformation to the isomeric form. These properties make phytochrome a unique photoreceptor and raise important questions concerning the function of phytochrome in plants growing in the natural environment[3,4]. In the physiological experiments which have been so successful in elucidating the details of the structure and properties of the molecule, and which are beginning to provide evidence on its cellular mode of action, aetiolated plants are usually used and are given abnormal irradiation treatments involving brief or prolonged exposure to radiation of restricted wavelengths. Such conditions do not occur in the natural environment and, therefore, such experiments provide no evidence on the role of phytochrome in plants growing in the field. Suggestions have been made that phytochrome may enable the plant to detect shading by other plants and thus induce the alteration of metabolism and development in an appropriate manner[5,6]. These proposals point out that radiant energy below 700 nm is almost completely reflected or absorbed by vegetation, whilst that between 700–800 nm (that is, the far red) is largely transmitted[7-10]. The relative enhancement of the far red will presumably alter the photoequilibrium of $P_r \rightleftharpoons P_{fr}$ in plants growing under vegetation canopies, presenting a possible mechanism for the detection of shading. No systematic attempts have yet been made, however, to determine the extent of the spectral energy changes in the natural environment, to correlate them with effects on phytochrome photoequilibria and to assess the possible regulatory role of phytochrome under these conditions. In this report we present preliminary evidence along these lines which is consistent with the above hypothesis.

Measurements of the spectral energy distributions (SED) of natural radiation between 400 and 800 nm were made using a spectroradiometer (Gamma, San Diego) equipped with a

flexible fibre-optic light guide and a remote miniature cosine-corrected receptor head. The instrument scanned the wavelength range in 40 s and the receptor head could be placed in any position within and outside crop canopies without disturbing the vegetation. The scanned spectra were corrected for instrument response by calibration against standard lamps (National Physical Laboratory, Teddington, UK). Phytochrome photoequilibria were determined by exposing freshly prepared aetiolated bean (*Phaseolus vulgaris*) hypocotyl hook sections in a plastic cuvette to the various natural and artificial radiation sources. The cuvette was cooled to 0 °C in an ice bath and exposed for a sufficient period of time for photoequilibrium to be reached. The P_{fr}/P_{total} proportions were then determined in a modified Perkin Elmer 156 dual wavelength spectrophotometer using standard procedures[11,12]. All P_{fr}/P_{total} data presented are the means of at least 12 determinations.

Table 1 Photoequilibrium dependence on $E_{660}:E_{730}$ ratio

	$E_{660}:E_{730}$	P_{fr}/P_{total}
Midday daylight	0.99–1.17	0.59–0.62
Wheat canopy	0.21	0.33
Sugar-beet canopy	0.033–0.045	0.06–0.10
Incandescent	0.71	0.55
Fluorescent	13.5	0.76
Red	20.1	0.80
Far red	0.002	0.04

The values for the wheat canopy are the means of measurements taken at ground level on June 27, 1974. For sugar-beet, a typical range of values are presented from measurements in the dense shade below one or more leaves on June 11, 1974. Red light was produced by filtering the light from eighteen 2 foot × 20 W red fluorescent tubes through two layers of No. 14 ruby Cinemoid (Rank Strand Electric, London). Far red light was produced by filtering the light from 40 150 W incandescent bulbs through two layers of No. 5A deep orange and two layers of No. 20 deep blue (primary) Cinemoid.

In a series of measurements made throughout the growing seasons of 1973 and 1974, only slight variation was observed in the SED of natural daylight; more was seen in daylight filtered through wheat and sugar-beet crop canopies. 'Typical' midday SED scans of natural daylight and canopy light (that is, daylight filtered through a vegetation canopy) are shown in Fig. 1. The very large relative enrichment of far red wavelengths in canopy light is apparent. When seeking to correlate changes in SED with phytochrome photoequilibria, it is useful to have a single parameter to describe the SED data. The simplest, though possibly not the best, is the ratio of absolute energy, in quantum terms, of the wavelengths of maximum absorption of P_r and P_{fr}, that is, $E_{660}:E_{730}$. It is possible to derive this value from the spectroradiometer scans, and we have been able to show that terrestrial plants normally exist under conditions in which the $E_{660}:E_{730}$ ratio ranges from about 1.2 in full sunlight, to about 0.05 in dense vegetation.

Using a variety of characterised natural and artificial radiation sources providing $E_{660}:E_{730}$ values from almost 0 to 20 we have
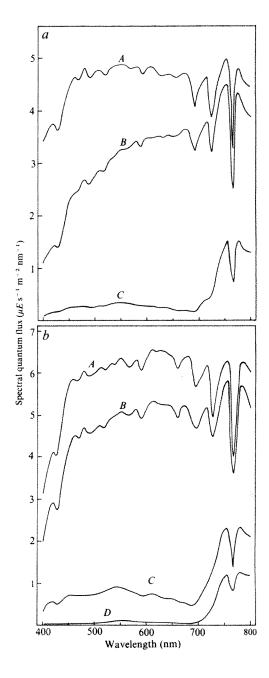
Fig. 1 SED of daylight above and below two crop canopies. *a*, Winter wheat canopy with a partially clear sky measured between 1000 and 1020 GMT on June 14, 1974: *A*, above canopy; *B*, ground level in sunfleck; *C*, ground level in shade. *b*, Sugar-beet canopy with a partially clear sky measured between 1320 and 1335 GMT on August 6, 1974: *A*, above; *B*, ground level between rows; *C*, and *D*, ground level within rows.

Table 2 Developmental changes in *T. maritimum* and *C. album* over 15 days at 25 ± 1 °C under fluorescent (low far red) and incandescent (high far red) light sources

	T. maritimum		*C. album*	
	Fluorescent	Incandescent	Fluorescent	Incandescent
Height (cm)	29.7±1.2	59.4±1.4	15.0±0.9	28.4±1.5
Internode length (cm)	0.84±0.09	3.53±0.18	—	—
Leaf dry weight (g)	0.483±0.029	0,464±0.024	0.338±0.016	0.310±0.016
Stem dry weight (g)	0.329±0.017	0.583±0.032	0.104±0.006	0.197±0.009
Leaf: stem dry weight ratio	1.47	0.80	3.25	1.57
Leaf area (cm²)	—	—	107.1±1.2	78.5±0.9
Chlorophyll $a+b$ (mg per g fresh weight)	75.7±1.9	67.0±1.7	112.3±1.8	93.8±4.5

The light sources were adjusted to produce equal quantum fluxes in the 400–700 nm waveband. *T. maritimum* seedlings were grown in a glasshouse until 9 weeks old, then transferred to fluorescent lighting in a growth chamber for 1 week before starting the treatments which were carried out in 8-h days. *C. album* were collected from an open situation in the field, potted and grown under fluorescent lighting for 10 d. The plants were then divided equally between the fluorescent and incandescent sources; 16 h photoperiods were used.

attempted to correlate SED to phytochrome photoequilibria. The results are shown in Table 1 and illustrate that natural conditions (E_{660}:E_{730} from 0.05 to 1.2) provide P_{fr}/P_{total} values ranging from near 0 to about 0.6. The theoretical maximum P_{fr}/P_{total} is 0.81, resulting from the overlapping absorption spectra of P_r and P_{fr}. A relatively small change in E_{660}:E_{730} causes a substantial change in P_{fr}/P_{total}, indicating that phytochrome may be a highly sensitive detector of SED in the red and far-red regions.

Although the typical values of photoequilibria presented here follow a predictable relationship to actinic flux quality, it would be incorrect for several reasons to predict actual P_{fr} levels in plants grown in light from these data. First, differential attenuation of light by masking pigments will alter intracellular light regimes. Second, the molecular environment of phytochrome is an important factor in determining the photoequilibria, and this cannot be assumed to be identical in aetiolated and light-grown tissues[13]. Third, a substantial proportion of phytochrome may be in the form of phototransformation intermediates under conditions of natural irradiation[14]. There are no methods presently available, however, which allow the determination of phytochrome photoequilibria in green tissue.

The crucial question is whether or not the changes in phytochrome photoequilibria which we suspect occur in the natural environment actually control development. To test this, various species are being grown for prolonged periods under artificial irradiation conditions which give equivalent rates of photosynthesis, but have widely different E_{660}:E_{730} ratios. Preliminary data of the effects of a fluorescent source and an incandescent source on the development of two common arable weeds are presented in Table 2. The light sources provided equal quantum fluxes in the 400–700 nm photosynthetically active region, but established different phytochrome photoequilibria (fluorescent source, 0.76 P_{fr}/P_{total}, and incandescent source, 0.55).

The pattern of development under the two sources is clearly very different, with the source providing lower P_{fr}/P_{total} causing markedly increased internodal elongation and reduced leaf size. It should be borne in mind that the photoequilibria under the incandescent source was only marginally lower than that in normal summer daylight, whereas that under the fluorescent source was higher than values ever observed in nature. It will therefore be necessary to carry out the technically much more difficult exercise of constructing irradiation sources giving high photosynthetic rates but very low P_{fr} levels before definitive growth experiments can be carried out. Even so, the striking developmental effects of the small reduction of the P_{fr}/P_{total} from 0.76 to 0.56 are consistent with the view that phytochrome acts to perceive the changed quality of natural radiation resulting from shading by other plants. On the basis of this admittedly circumstantial evidence we support the working hypothesis that the function of phytochrome is to enable the plant to react appropriately to shading. This property would be of undoubted adaptive value and would ensure the persistence of phytochrome throughout evolution.

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Male sterility in wheat plants deficient in copper

In higher plants, copper deficiency affects the reproductive phase more than the vegetative. Yields of grain may be markedly reduced or nil without much effect on yield of vegetative parts[1-3]. Failure to produce seed may be caused by lack of sufficient photosynthate production or translocation, or to the absence of fertilised embryos. Although soluble carbohydrates were low in copper-deficient wheat leaves, the evidence reported here points to the non-viability of pollen as the primary cause of failure to set grain. Possible mechanisms for the induction of male sterility by copper deficiency are proposed, and its potential for use in plant breeding is discussed.

Copper-deficient wheat plants developed small anthers, commonly less than half as long as those of normal plants and of about one-tenth the volume (Fig. 1). Pollen grains were fewer in number, small, and often dented like deflated footballs (Fig. 1). Copper-deficient pollen did not stain with iodine-potassium iodide solution (Table 1 and Fig. 1), indicating non-viability[4].

Table 1 Effect of copper on pollen production and viability in wheat

Copper status of plants	Pollen grain numbers per anther	Stained pollen grains (%)
Copper-deficient	76	0
Copper-sufficient	3,400*	95

Values are the means for nine anthers.
*This value was not determined in this study but from counts of pollen grains in four anthers of healthy plants of the same cultivar in another study.

Cross pollination experiments (Table 2) confirmed the non-viability of copper-deficient pollen and also showed the viability of the ovule. With the exception of two grains in one head, no seed was set from copper-deficient pollen; in contrast, pollen from copper-sufficient plants set grain with both copper-sufficient and copper-deficient ovules. In copper-deficient ovules, normal pollen set grain in 30% of the florets. Most ovules may have been viable since pollen was applied only once, and fertile florets were found in all parts of the heads.

Time-of-application studies showed that grain set from deficient plants responded dramatically when copper was applied until 12 weeks after sowing, after which there was no response, except in later tillers. The critical period in copper-deficient plants was the early boot stage, which approximates to meiosis in microsporogenesis. Synchronised meiotic divisions in a large number of pollen mother cells[5] may produce a localised demand for copper exceeding that which the deficient plant can supply. This hypothesis could be tested by studying

Table 2 Grains set by cross pollination between copper-deficient and copper-sufficient wheat plants

Cross ♀ × ♂		No. of grains set	Total no. Florets	Heads
CD	CD (selfed)	0	76	3
CS	CD	2	76	3
CD	CS	47	157	7
CS	CS (selfed)	86	86	3

The values for the selfed heads are typical of many heads produced during these studies.
CD, copper-deficient; CS, copper-sufficient.

Fig. 1 *a*, Florets excised from copper-deficient (left) and copper-sufficient wheat plants. The copper-deficient anthers are much smaller than normal and the ovule is slightly smaller; one anther is still attached to the receptacle. The filaments do not elongate. (× 5). *b* and *c*, Pollen grains (treated with iodine) from copper-deficient (*b*) and copper-sufficient (*c*) wheat plants. The copper-sufficient grains stain strongly and are 55–60 μm in diameter; copper-deficient grains are 40–45 μm in diameter and do not stain. Wheat plants (*Triticum aestivum* cv. Halberd) were grown in a copper-deficient siliceous sand from Tintinara, South Australia (Laffer sand) in an evaporatively cooled glasshouse. Each pot contained 12 kg of soil which supported three plants sown on July 2, 1974. Appropriate amounts of nutrients N, P, K, Ca, Mg, S, Fe, B, Mn, Zn, Mo and Cl were added with or without Cu (at 4 mg per pot). Plants supplied with copper grew normally while copper-deprived plants showed typical deficiency symptoms: wilting, wither-tip, rat-tailed ears, delayed maturity and failure to set grain (first reported by Lipman and Mackinney[1]).

meiosis in relation to the time of application of copper, and if confirmed, would explain why a supply of copper, just sufficient for vegetative growth, may be inadequate for the reproductive phase.

An alternative hypothesis is that non-viability is caused by the lack of accumulation of starch in the developing pollen. This could be related to low soluble carbohydrate levels in copper-deficient plants (R. D. G., unpublished). Because the responsiveness to copper changes quite suddenly, however, it seems that the effect is occurring at a more critical stage of pollen formation than starch accumulation.

These findings may assist cytological and biochemical studies of microsporogenesis as well as being of potential practical use for the production of hybrid seed. It should prove possible in reproducible and well defined low copper environments, both in the field and in the glasshouse, to grow wheat, and probably other species, which are consistently male sterile. This would be especially useful where emasculation is unusually difficult or time-consuming.

Environmental conditions such as moderate water, temperature or nutritional stress can induce male sterility in genetically normal plants. Copper deficiency is a good example, and similar responses to boron deficiency have been reported[6].

This type of plant response may be important in their evolution as, by the increased possibility of cross pollination caused by male sterility in a harsh environment, adaptation would be more rapid.

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Elimination of mycoplasmas from cell cultures with sodium polyanethol sulphonate

MYCOPLASMAS are common contaminants of cell cultures, though rarely of primary cultures[1–3]. The organisms can have a variety of effects on cells in cultures[5]. They can severely interfere with the metabolism of the cells. Depletion of the culture medium content of arginine causing chromosomal changes and morphological alteration of the cells have been observed, although cytopathogenic changes similar to those caused by viruses are less common. The use of cell cultures contaminated with mycoplasmas may supress the yield of viruses, but occasionally enhance it. Since some species of *Mycoplasma* may haemadsorb and haemagglutinate, the presence of such organisms in cell cultures may be mistaken for that of certain viruses.

Methods to eradicate mycoplasmas from cell cultures[3] are usually of restricted value, since the organisms, although not detectable for some time, usually reappear. Such methods include treatment of the cultures with antibiotics[6], and/or antisera homologous to the contaminating species of *Mycoplasma*[3]. Heating of the cultures at 41 °C or treatment with N-tris-(hydroxymethyl)methylglycine (TRICINE) buffer has also been reported[7]. An intracellular localisation of the mycoplasmas would explain why contaminated cell cultures are refractory to treatment, but it is not yet known whether mycoplasmas do occur intracellularly.

Sodium polyanethol sulphonate (SPS) is an anticoagulant, which also inhibits the bactericidal activity of serum[8,9], and is therefore used in transport media for blood specimens to be cultured for bacteria. A 5% solution of SPS impregnated on paper disks inhibits the growth on solid medium of the vast majority of species of *Mycoplasma*, but not those of *Acholeplasma*[4]. SPS (Koch-Light) was used for eliminating mycoplasmas contaminating cell cultures of the following contaminated cell lines. Human embryonic lung fibroblasts (HEL), HeLa (strain S3), MASA (originally derived from bone marrow[10]) and McCoy cells.

SPS (10%) was sufficient to eradicate the mycoplasmas from the HeLa and McCoy cell lines after incubation for 22 h (Table 1). The cultures remained free of mycoplasmas within the 4 months of follow-up. The HEL cells were more susceptible to SPS and did not survive treatment for 6 h with a 5% solution. Lower concentrations of SPS were not enough to kill the mycoplasmas in the HEL cell cultures. As for the MASA cells, the mycoplasmas could not be eradicated by one treatment with SPS in concentrations which did not also kill the cells. The mycoplasmas were, however, reduced by 10^4 colony-forming units (CFU ml^{-1}) in the culture medium by the first treatment. After treated a second time with 10% SPS for 6 h the cell cultures were free from mycoplasmas and remained so for at least 4 months.

Eradication of the mycoplasmas was successful only when the culture bottle was completely filled with the mixture of the

Table 1 Susceptibility of some cell lines and of contaminating mycoplasmas to SPS

Cells	SPS (%)	Period of treatment (h)	Myco-plasmas eliminated	Cell line killed
HeLa	5	22	—	—
	10	22	+	—
McCoy	5	22	—	—
	10	22	+	—
HEL	2	6	—	—
	5	6	NT†	+
MASA	5	6	—	—
	10	6	—*	—
	10	22	NT†	+

*The treatment reduced the number of mycoplasmas in the culture medium with 10^4 CFU ml^{-1}. Repeated treatment with 10% SPS for 6 h after the cells had been passaged, eliminated the mycoplasmas completely from the cell culture for at least 4 months.

†Not tested.

Cells were grown in MEM (Flow) with foetal calf serum (final concentration 10%), and propagated with the aid of versene. Before and after treatment with SPS, cultures were tested for mycoplasmas in serial dilutions of the tissue culture medium in liquid mycoplasma medium, and by direct inoculation on to agar plates[11]. The culture medium specimens tested also contained cells. In some instances, the cell suspensions were also frozen and thawed before culture for mycoplasmas. Mycoplasmas isolated from the cultures before treatment belonged to the *M. orale* (type 1) species. The culture medium contained at least 10^6 colony-forming units (CFU) of *Mycoplasma* ml^{-1} before treatment with various concentrations of SPS, at which time they produced a confluent layer of cells in Carrel bottles. The mixtures were incubated at 37 °C for various periods after which the cells were passed to new bottles. Attempts were made to isolate mycoplasmas before each new passage of the cells. If not stated otherwise, the results presented are based on those obtained from a single treatment with SPS.

culture medium and the SPS. The treatment also killed a large proportion of the cells, but did not disturb further propagation of the cell lines. Cells cultured in suspension proved more susceptible to SPS than when the corresponding cells were cultured in monolayer.

In many laboratories the majority of cell lines used are presumably contaminated with mycoplasmas. We found mycoplasmas in more than 60% of a series of 400 specimens of cell culture media from such cultures. The route of infection of cell cultures with mycoplasmas is often obscure, although contamination of the cultures by mycoplasmas from the respiratory tract of the persons handling the cultures and the use of contaminated constituents of the cell culture medium, particularly serum, have been considered the commonest sources of infection. The former may be suspected for the cell cultures we studied, since the mycoplasmas isolated belonged to the species of *M. orale* (type 1).

The mechanism by which SPS kills mycoplasmas is not known, but it is probably by lysis. The presence of cholesterol in the cytoplasmic membrane of mycoplasmas but not that of acholeplasmas would explain the difference in susceptibility to this compound, the former organisms being more susceptible. When tested with the disk-diffusion technique a 5% solution of SPS did inhibit mycoplasmas, with the exception of certain strains of *M. anatis*, *M. gallinarum*, *M. gatae*, *M. iners* and *M. maculosum*, but not acholeplasmas[4]. The mycoplasmas isolated from the cell cultures we studied belonged to one species, *M. orale* (type 1). The susceptibility to SPS of the great number of other species of *Mycoplasma* known to contaminate cell cultures is to be tested. The susceptibility of *M. orale* (type 1) does not seem to differ, however, from that of the majority of other species of *Mycoplasma*[4]. The presence of cholesterol in the cytoplasma membrane of mycoplasmas and its absence in that of acholeplasmas has also been offered as an explanation of the differential effect of lysolecithin on these organisms[12]. We also tested whether lysolecithin could

be used for eliminating mycoplasmas from cell cultures, but the differential between the concentrations that killed the mycoplasmas and the cells proved too small for lysolecithin to be of use for this purpose, at least for the cell lines studied. This also seems to hold for certain cell lines with SPS.

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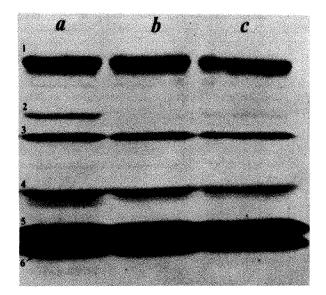
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Penicillin-binding proteins and cell shape in *E. coli*

β-LACTAM antibiotics (penicillins and cephalosporins) have attracted considerable attention as probes of cell growth and division[1,2]. Although extensive studies have been made on both penicillin-sensitive enzymes and penicillin binding proteins[3], there has been no clear indication of the role of any of these components in the effects of β-lactam antibiotics on cell growth. We report the identification of a minor penicillin binding protein which we believe to be the target at which the amidinopenicillanic acid designated FL1060 (ref. 4) acts to affect the shape of *Escherichia coli*. This is the first example of the identification of a penicillin binding protein with an essential and defined role in bacterial cell growth.

Whereas low concentrations of typical β-lactam antibiotics specifically inhibit cell division in *E. coli*, FL1060 at its lowest effective concentration causes the conversion of *E. coli* rods into ovoid shaped cells[4,5]. The atypical effects of FL1060 are paralleled by its failure markedly to inhibit any of the three known penicillin-sensitive enzymes of *E. coli*[5,6] and by its apparent failure to compete with the binding of ^{14}C-benzyl-penicillin to *E. coli* membranes[6]. As these experiments measured total enzyme, or total binding, it is still possible that FL1060 inhibits a minor enzyme species or competes with a minor penicillin-binding protein in the cell envelope. We have re-examined the binding of FL1060 to the *E. coli* penicillin binding proteins using an improved method for the detection of the individual binding proteins, and have shown that FL1060 does indeed compete with high affinity for a minor penicillin binding protein in the *E. coli* inner membrane.

^{14}C-benzylpenicillin (54 mCi mmol^{-1}; Searle Amersham) was bound to purified membranes of *E. coli* K12 (strain KN126 obtained from Dr T. Nagata). The membrane proteins were solubilised and fractionated on sodium dodecyl sulphate (SDS) polyacrylamide slab gels. As conventional autoradiography was too insensitive to allow rapid detection of the binding proteins, they were detected by incorporating the scintillant 2,5,-diphenyloxazole (PPO) into the gel matrix (fluorography[7]) and exposing the dried gel to Kodak RPRoyal X-ray film at -70 °C (ref. 7). Figure 1a shows the pattern of six penicillin-binding proteins consistently obtained from the membranes of *E. coli* K12. As all six proteins are found exclusively in the inner (cytoplasmic) membrane (our unpublished results) we routinely removed the outer membrane after binding ^{14}C-benzylpenicillin (taking advantage of the insolubility of the outer membrane in 1% Sarkosyl at room temperature[8]) and fractionated only the

Fig. 1 Competition of FL1060 and 6-APA for penicillin-binding proteins. Membranes were prepared from *E. coli* KN126 growing exponentially in Difco antibiotic medium No. 3 as described previously[9], washed twice in 0.01 M sodium phosphate buffer, *p*H 7.0, and resuspended at 20 mg ml^{-1} in 0.05 M sodium phosphate buffer, *p*H 7.0; 10 mM MgCl$_2$. To assay penicillin binding proteins 20 μl of ^{14}C-benzyl-penicillin (50 μCi ml^{-1}, specific activity, 54 mCi mmol^{-1}) was added to 200 μl of washed membranes for 10 min at 30 °C. The final concentration of benzylpenicillin was 34 μg ml^{-1}, sufficient to saturate the binding proteins in 10 min at 30 °C. The reaction was stopped and the inner membrane was solubilised by addition of 10 μl of 20% (w/v) Sarkosyl (Geigy) and 5 μl of cold benzylpenicillin (120,000 μg ml^{-1}). After 20 min at room temperature the samples were centrifuged at 100,000*g* for 40 min and 100 μl of the supernatant was added to 50 μl of SDS buffer (0.2 M Tris-HCl, *p*H 6.8; 3% SDS; 30% glycerol; 0.002% bromophenol blue). After addition of 15 μl of β-mercaptoethanol, the samples were heated at 100 °C for 2 min and 50 μl was loaded on a 12% discontinuous SDS polyacrylamide slab gel using the buffer system and apparatus described by Laemmli and Favre[10]. After fixing the gel, the scintillant PPO was incorporated into the gel and the binding proteins were detected by fluorography[7] on Kodak RPRoyal X-ray film for 23 d at −70 °C. *a*, Membranes preincubated with 10 μl of distilled water for 10 min at 30 °C. *b*, Membranes preincubated with 10 μl of FL1060 (100 μg ml^{-1}) for 10 min at 30 °C (final concentration of FL1060 was 5 μg ml^{-1}). *c*, Membranes preincubated with 10 μl of 6-APA (1 mg ml^{-1}) for 10 min at 30 °C (final concentration of 6-APA was 50 μg ml^{-1}.) Electrophoresis was from the top to the bottom of the figure.

Sarkosyl-soluble inner membrane. As radioactive FL1060 is not readily available, we have studied the binding of FL1060 to the penicillin binding proteins by competition with ^{14}C-benzylpenicillin. Figure 1*b* shows that prebinding FL1060 (5 μg ml^{-1}) for 10 min at 30 °C resulted in the complete blocking of the subsequent binding of ^{14}C-benzylpenicillin to binding protein 2 without affecting any other binding protein. A more detailed analysis showed that FL1060 (0.013 μg ml^{-1}) produced a 50% inhibition of the binding of a saturating concentration of ^{14}C-benzylpenicillin to binding protein 2. This is in good agreement with the minimal inhibitory concentration (MIC) of 0.05 μg ml^{-1} required to inhibit the growth of this strain. A similar blocking of binding to protein 2 was observed if ^{14}C-benzylpenicillin was added to membranes prepared from cells grown for 60 min in the presence of FL1060 at 0.1 μg ml^{-1}.

To implicate binding protein 2 further in the effects of FL1060 on cell shape we screened a number of penicillins and cephalosporins to find those which produced similar morphological effects to those produced by FL1060. Among the compounds screened, only the penicillin precursor molecule 6-aminopenicillanic acid (6-APA) resulted in the production of ovoid cells at the lowest effective concentrations, and at no concentration did it produce the specific inhibition of cell division typical of most β-lactam antibiotics. 6-APA was

markedly inferior to FL1060 in its action on cell shape as it was only effective at relatively high concentrations (MIC 14 μg ml^{-1}) and over a narrow concentration range above which lysis occurred. Figure 1*c* shows that as expected 6-APA at 50 μg ml^{-1} completely inhibited the binding of ^{14}C-benzylpenicillin to binding protein 2. A concentration of 2 μg ml^{-1} 6-APA produced 50% inhibition of binding to protein 2. Significant inhibition of binding proteins 1, 3 and 4 was also produced by 6-APA at 50 μg ml^{-1}. This is probably the cause of cell lysis by 6-APA as the binding of β-lactams to these latter proteins is thought to be the cause of the effects of typical β-lactam antibiotics on cell growth and division (our unpublished results).

Binding protein 2 has an apparent molecular weight of 66,000 as measured by its relative mobility on SDS polyacrylamide slab gels compared with that of 7 standard proteins, and constitutes 0.5% of the total penicillin binding at saturating concentrations of ^{14}C-benzylpenicillin. This represents about 10 copies of binding protein 2 per cell. Typical β-lactam antibiotics that affect cell division and cause cell lysis either fail to compete with binding protein 2 (for example, most cephalosporins) or bind with low affinity. These latter antibiotics do not result in the production of ovoid cells as they bind to other proteins thought to be involved in cell division and cell elongation with greater affinity than they do to binding protein 2 (our unpublished results).

We believe that penicillin-binding protein 2 is the target at which FL1060 acts to affect the shape of *E. coli*. The protein is presumably an enzyme involved in the terminal stages of peptidoglycan metabolism. At present it is not clear whether this is a previously unknown enzyme or a minor component of one of the established penicillin-sensitive enzymes[3]. As FL1060, unlike typical β-lactam antibiotics, is much more effective against Gram-negative than Gram-positive organisms it has been suggested that it acts on a target which is unique to the former class of organisms[3] (R. James, J. Haga and A.B.P., unpublished).

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Immunological and bacteriological basis for vaccination against dental caries in rhesus monkeys

A REPRODUCIBLE model for dental caries has been established in rhesus monkeys[1]. The development of caries is comparable to that found in man because caries is induced by maintaining the animals on a human type of diet, this is associated with an overgrowth of a naturally acquired *Streptococcus mutans* and caries develops in teeth that are morphologically similar to human teeth. The incidence of

smooth surface caries was reduced by subcutaneous or submucous immunisation with a heat-killed *Strep. mutans* in Freund's incomplete adjuvant (FIA)[2]. Protection has been correlated predominantly with the rate of development of serum complement fixing antibodies to a hydroxyl apatite fraction of the culture supernatant (HACS) of *Strep. mutans*. The aims of this investigation were to attempt to elucidate the mechanism of prevention of dental caries in rhesus monkeys by immunisaion with a monkey passaged *Strep. mutans*. The relative effects of saliva and crevicular fluid on *Strep. mutans* were tested by developing a differential sampling technique across the plaque and the adjacent saliva and crevicular fluid. This revealed that the very low caries score in the immunised animals was correlated with serum antibodies and with a reduction in the number of *Strep. mutans* in crevicular fluid and the adjacent bacterial plaque zone.

A series of 11 young rhesus monkeys were caged, examined and maintained on a human type of diet, containing 15% sucrose as described previously[1]. All animals had a fully erupted deciduous dentition but no permanent teeth and their ages were assessed to range from 11 to 21 months (ref. 3). The animals were examined at monthly intervals and a steady increase in weight was recorded in all animals.

A streptomycin resistant *Strep. mutans* (serotype c) was successfully implanted into the mouth of another rhesus monkey, then reisolated and a heat-killed vaccine containing about 10^9 of the passaged *Strep. mutans* per ml was prepared and used as described previously[2]. The animals were randomly distributed into three groups and there was a comparable sex distribution in each of these groups. One

group of three monkeys was given the first subcutaneous injection of 0.5 ml of the vaccine in an equal volume of FIA into the contralateral limbs. This was followed by four injections of 1 ml of the vaccine alone at weeks 4, 8, 16, and 56. The adjuvant control group of four animals was given subcutaneous injections of 1 ml of FIA, followed by four saline injections; the saline control group of four animals was given five subcutaneous injections of saline at the same time intervals.

Blood was taken from the femoral vein at monthly intervals and always before immunisation, and the serum was stored at $-20\,°C$. The blood indices remained within the normal range throughout the investigation. Serum antibodies were assayed by the complement fixation (CF) test in microplates, as described previously[4]. The immunised group showed a rapid increase in CF antibodies from a mean baseline titre of $\log_2$ 1–4 within four weeks and reached a maximum of $\log_2$ 6 by week 24 (Fig. 1). A small decrease in titre followed, but the booster injection at week 56 induced a secondary antibody increase to $\log_2$ 6. Both control groups had a very low serum antibody titre of less than $\log_2$ 3.

Whole saliva was collected at monthly intervals, after subcutaneous injection of 0.5 mg per kg of pilocarpine[1], and the haemagglutinating antibodies were determined by a modified micromethod[4]. The immunised group showed a modest increase from a mean titre of $\log_2$ 1.2–2.3 and remained between $\log_2$ 2–3 from week 4 to 84. Both control groups showed little initial change but from week 24 to 48 titres between $\log_2$ 1 and 2 were found in the saline group and between $\log_2$ 2 and 3 in the adjuvant group.
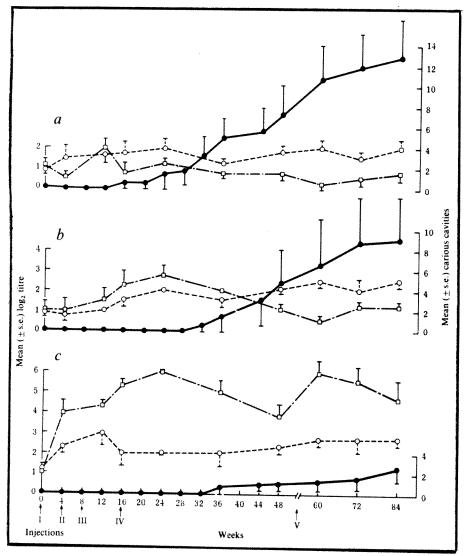
Fig. 1 Sequential serum and salivary antibody responses to immunisation and caries score over a period of 84 weeks. *a*, Saline group; *b*, adjuvant group, *c*. *Strep. mutans*/adjuvant group. ●, Caries index; □, serum antibodies; ○, salivary antibodies.

Clinical and X-ray examination for dental caries was performed at monthly intervals and caries was scored as described previously[1]. The mean onset of smooth surface caries was 28.5 ($\pm$4.6) weeks in the saline group, 54.5 ($\pm$10.8) weeks in the adjuvant group and more than 55 weeks in the immunised group (one animal had no caries by week 84). Both control groups showed a rapid increase in smooth surface caries (Fig. 1) and by week 84 a mean of 12.75 ($\pm$3.6) cavities per animal was found in the saline group and 9.0 ($\pm$4.3) cavities in the adjuvant group of monkeys. In contrast the immunised group had developed only 2.7 ($\pm$1.4) cavities per animal. The results suggest that immunisation had delayed the onset of caries and had decreased both the rate of development and the incidence of caries. The serum CF antibodies seemed to be associated with this protection, as was found previously[2]. Fissure caries appeared from week 20 in the saline and week 28 in the adjuvant group; by week 84 there were 2.0 ($\pm$1.3) and 2.5 ($\pm$1.0) cavities per animal respectively. Fissure caries was not found in the immunised group by week 84.

No attempt was made to implant *Strep. mutans* into any of the animals. A sequential analysis of randomly pooled plaque, from the buccal and approximal surfaces of teeth was carried out at monthly intervals from 0 to 6 months. Samples of plaque were placed into transport medium and then cultured, under anaerobic conditions, on TYC medium[5]. *Strep. mutans* and *Strep. sanguis* were identified by their colonial morphology, production of dextran and carbohydrate fermentation reactions. *Strep. mutans* (serotype c) was isolated from dental plaque from week 4 and all animals harboured a large number of these *Streptococci* from week 24 to 84. The mean time of isolation of *Strep. mutans* from the saline group was 15.5 ($\pm$5.0) weeks, from the adjuvant group 19.5 ($\pm$1.5) weeks and from the immunised group 20 ($\pm$2.0) weeks. In contrast, *Strep. sanguis* was isolated from all animals between 0 and 4 weeks and persisted throughout the experimental period.

Once the *Streptococci* were established in all animals, a quantitative analysis was performed, to find out if immunisation had any effect on colonisation by *Strep. mutans*. Weighed samples of plaque were collected in 2 ml of transport medium, at monthly intervals, from 6 to 12 months. The samples were dispersed by shaking vigorously with glass beads and then plated out in serial tenfold dilutions on TYC medium. The results were expressed as a percentage of colony forming units (CFU) of *Strep. mutans* and *Strep. sanguis*, in terms of the total number of *Streptococci* per mg of plaque grown on the TYC medium. Little difference was found between the mean CFU of *Strep. mutans* of saline injected (67 $\pm$7.7), adjuvant treated (46$\pm$9.4) and immunised (54 $\pm$15.0) animals. In view of this and the fact that serum and not salivary antibodies seem to be associated with protection against caries both in rhesus monkeys[2] and in man[4], the question was posed whether protection was mediated by serum through crevicular fluid. The hypothesis to be tested was that according to the immune state of the animal there should be a quantitative difference in *Strep. mutans* in the 4 zones adjacent to the tooth (Fig. 2); crevicular fluid, crevicular fluid plaque and salivary plaque zones and saliva.

An aliquot of 0.1 ml of the whole saliva was placed directly into transport medium. The salivary plaque zone was collected by lightly removing with a probe all plaque from about the cervical third of the buccal surfaces of the left maxillary molars and placing this into transport medium. The 'crevicular fluid-plaque zone' was sampled by rubbing the blunt end of a sterile root canal paper point against the surface from which the 'salivary-plaque zone' had just been removed and cutting off the end of the paper point into transport medium. Finally, crevicular fluid was collected from the same site by placing the needle of a 50 µl syringe between the two deciduous molars and flush-
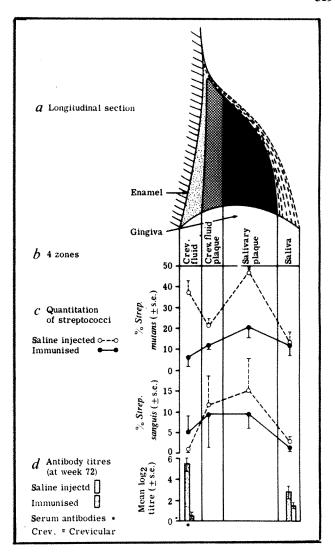
Fig. 2 Salivary and crevicular fluid immune mechanisms affecting *Strep. mutans* in the four zones adjacent to the tooth.

ing through and immediately aspirating 10 µl of transport medium[6]; this was repeated and the washings were collected into transport medium. The four specimens were suitably diluted and the CFU of *Strep. mutans* and *Strep. sanguis* were determined monthly between 12 and 18 months. The results were expressed by comparing the sum of the means ($\pm$s. e.) of the six serial determinations in each animal in one group with those in another group (Fig. 2) Although three of the four sampling sites showed a lower percentage of *Strep. mutans* in the immunised than control groups this reached significant levels only in crevicular fluid (t=4.035; P<0.02) and the crevicular fluid–plaque zone (t=2.77; P<0.05). The results for the adjuvant group were intermediate between the saline and immunised groups. A significant difference in the corresponding results with *Strep. sanguis* was not found; indeed there were about nine times more *Strep. sanguis* in the crevicular fluid of immunised than saline injected animals. It is also of considerable interest that the crevicular fluid of saline injected animals yielded about 60 times more CFU of *Strep. mutans* (38) than *Strep. sanguis* (0.6), whereas the corresponding ratio in the immunised animals was about 1:1, or 6 CFU in each group. Preliminary determinations of the percentage of *Strep. mutans* in terms of the total anaerobic bacterial growth on blood agar showed that crevicular fluid of the saline injected group yielded about 25 times more *Strep. mutans* than the immunised group.

There are two mechanisms which may be involved in

prevention of caries: first salivary IgA preventing adherence of *Strep. mutans*[7], and second, crevicular fluid mediated serum antibodies and blood leucocytes inhibiting *Strep. mutans* or its products, suggested here. The relative roles of saliva versus crevicular fluid were put to the test by developing a differential sampling technique across the plaque and the adjacent two fluid layers. This revealed that the very low caries score in the immunised animals was correlated with a low percentage of *Strep. mutans* in crevicular fluid and the crevicular fluid–plaque zone and an increased serum antibody titre to *Strep. mutans* (Fig. 2). A similar relationship was not found with salivary haemagglutinating antibodies and *Strep. mutans* in saliva and the salivary–plaque zone which probably constitutes the bulk of dental plaque as tested conventionally. A significant reduction in *Strep. mutans* in plaque of immunised animals was not found by others[8-10], with the exception of one out of seven groups of animals in one series.[10] It is assumed that the immune responses in crevicular fluid are mediated by serum antibodies and possibly leukocytes, but this will have to be tested directly on crevicular fluid. The bacteriological results are consistent with the hypothesis that the immune components of crevicular fluid are responsible for protection against smooth surface caries, and that crevicular fluid may act as a functional analogue of blood.

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Photopigment conversions expressed in receptor potential and membrane resistance of blowfly visual sense cells

IN spite of the fact that intracellular recordings from photoreceptor cells have been possible for many years and knowledge regarding the photochemistry of these cells has been steadily increasing, it is still unclear how resistance changes and potential changes of the cell membrane are mediated by photoconversion of the pigments located in that membrane. It is generally supposed that a link does exist between the photochemistry and the membrane phenomena, but evidence is sparse.

Recent evidence is provided by the work of Hochstein *et al.*[1] on the barnacle and Minke *et al.*[2] on *Limulus* who demonstrated that light-evoked changes in resistance and potential of the receptor cell membrane depend on the photopigment conditioning. Their experiments are founded on two crucial facts in the photochemistry of these and many other invertebrate species. First, the photopigment has two thermostable states instead of one, and second, practically all pigment can be converted into either the rhodopsin or the metarhodopsin state when illuminated with carefully chosen wavelengths. It proved that stimulation with a metarhodopsin-generating wavelength of a receptor cell harbouring mainly rhodopsin, caused resistance and potential changes which persisted in the dark and could only be undone by a wavelength causing renewed rhodopsin production.
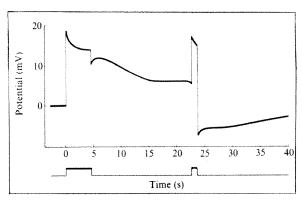
Fig. 1 Upper trace, membrane potential of a visual receptor cell (blowfly wild type) measured intracellularly with a glass microelectrode. Lower trace, light stimulus. Positive deflection represents membrane depolarisation. The cell has been preadapted to intense red light (603 nm). The first stimulus shown in the figure, delivered after 2 min in the dark, is a blue flash (460 nm), causing a depolarisation. Apart from a short dip, switching off the blue light does not change the light adapting course of the receptor potential. A flash of red light (second stimulus in the figure) depolarises the membrane again and induces normal repolarisation at its termination.

For the blowfly, the particulars of rhodopsin and metarhodopsin are given by Stavenga *et al.*[3,4] who described optical work on the pupil mechanism of this species. Their results point in the same direction as those of barnacle and *Limulus*, if their hypothesis of the pupil pigment granules migrating in an intracellular force field created by the resistance changes, (which are simultaneously giving rise to the receptor potential) is correct.

Hence, we have attempted to demonstrate in the blowfly a link between the photoconversions and the light-induced changes in resistance and potential of the photoreceptor membrane. The latter were measured intracellularly in the photoreceptor cells of practically intact specimens, both wild type and the mutant Chalky.

We used the following procedures (Fig. 1). First, stimulation with red light (603 nm) brought nearly all photopigment molecules into the rhodopsin state. After 2 min in the dark, a metarhodopsin-generating blue flash was then presented, inducing a normal receptor potential, that is, a depolarisation of the cell membrane. This was, however, not followed at its termination by a normal quick return to the preillumination level, that is, a repolarisation of the cell membrane. The very slowly repolarising cell membrane could then become depolarised again by a flash of intense red light which, on being turned off, promptly caused membrane repolarisation to the former dark level.

Such a recording of a blowfly wild type, is shown in Fig. 1,
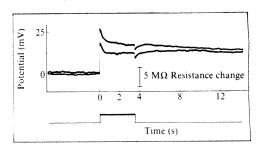
Fig. 2 Upper trace, membrane potential and resistance changes of a visual receptor cell (blowfly wild type). The resistance changes are measured by a square wave (30 Hz) driven bridge circuit. With a balanced bridge a single trace is seen, but it seems to split in two as soon as the bridge becomes unbalanced; the distance between the two "parallel" traces indicates the changed membrane resistance with respect to the preflash value, while the membrane potential is represented by their mean. Lower trace, light stimulus (blue light). The cell has been pre-adapted to red light (603 nm) and blue light is presented after 2 min in the dark. After the blue light is switched off neither the membrane potential nor the resistance returns to the preflash value. The dip in potential is not accompanied by a dip in resistance.

in which termination of the blue flash is marked by a dip in the receptor potential, which otherwise does not seem to be deflected from the slowly repolarising course which normally occurs during dark adaptation. We call this sustained depolarisation a 'tail', following Hochstein et al.[1]. At the end of the subsequent red flash a rapid repolarisation was induced which markedly exceeded the former dark level (Fig. 1).

We will now compare these data with those obtained for photoreceptor cells of the barnacle[2] and *Limulus*[3]. With an essentially similar stimulus programme, but different wavelengths (because of the different absorption spectra of rhodopsin and metarhodopsin in these species), Hochstein et al.[1] elicited from the barnacle visual sense cells, tails which differ only slightly from ours. The barnacle tails markedly deflect from the slowly repolarising course normal during light adaptation, by repolarising more rapidly. The blowfly tails are in fact also characterised by a change in repolarising rate, but in the other direction. In *Limulus*, Minke et al.[2] did not observe any change in repolarising rate.

At the beginning of the tail in the barnacle there is no dip as in blowflies, but a small repolarising step, while *Limulus* has an even smaller step. But apart from these small differences of tail-slope and dip-shape which may well be explained by the differences in photochemistry, it seems certain that the underlying mechanism must be very similar if not the same in the three species.

Following the proposal of Hochstein et al.[1] and Minke et al.[2], we suggest the following scheme for the blowfly. A great number of hypothetical particles called "excitors", capable of lowering the membrane resistance, are made by the blue flash as a byproduct of metarhodopsin. These excitors continue exerting their influence on the membrane resistance, thus creating a tail, without being hampered by hypothetical anti-particles called "inhibitors" which are normally present to restore the membrane resistance as soon as the light is switched off. As a result of our experimental procedure these "inhibitors" are lacking because they have all been made ineffective by the preceding 2 min of darkness.

This happens after every such dark period, regardless of the preceding illumination history. But without the special measures taken in our experiment, illumination after the 2-min dark period encounters a mixture of both rhodopsin and metarhodopsin and immediately starts the production of, respectively, metarhodopsin + excitor and rhodopsin + inhibitor, so that the membrane can repolarise as soon as the light is turned off. But as a result of our preceding intense red illumination, all metarhodopsin has been turned into rhodopsin + inhibitor, so after the 2-min dark period there is no metarhodopsin left for the manufacture of new inhibitors and the old inhibitors have all become inactive. Only after extended blue illumination, resulting in a new photopigment equilibrium, is there sufficient metarhodopsin to ensure continuous reversal to rhodopsin + inhibitor, resulting in normal restoration of the membrane resistance and repolarisation of the cell when the light is turned off.

The results of simultaneous recordings of the receptor potential and the membrane resistance changes are shown in Figs 2 and 3. The membrane resistance change should resemble the receptor potential unless an electrogenic pump is present. Figure 2 presents a detail of a similar experiment to that in Fig. 1. The receptor potential is accompanied by measurements of membrane resistance changes which were made with an a.c. square wave driven bridge circuit. After the initial depolarisation and resistance decrease at the onset of illumination, it is seen that during as well as after the blue flash the resistance increases again, even rather more than is necessary to keep pace with the slow repolarisation. The dip in the latter, at the beginning of the tail is not accompanied by a similarly temporary change in resistance.

Neither can this be observed in the recording shown in Fig. 3, which stems from a pupil-less blowfly mutant, the variety *Chalky*. In this particular animal the measured resistance chan-
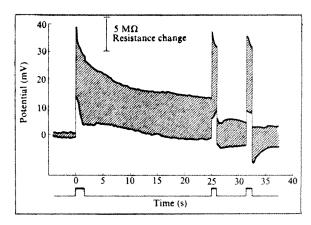
Fig. 3 As in Fig. 2. Measurements on a pupil-less blowfly, mutant *Chalky*. Blue light again causes a depolarisation and a decrease in membrane resistance and when the light is switched off, the cell again retains its light adapting course. Flashes of red light (second and third stimulus) depolarise the membrane as before and are followed by normal repolarisation while the resistance remains short of the former dark value.

ges were much greater than in the one represented in Fig. 2, but still, termination of the blue flash cannot be seen as a change in resistance, though again the dip in the receptor potential is present, even if rather small.

Yet another discrepancy between membrane potential and membrane resistance occurs when the tail is cut short by a red flash: at the cessation of the latter the receptor potential reaches or exceeds the former dark level, while the membrane resistance remains short of its former value.

At just this point a discrepancy also occurs in the barnacle, but here the resistance value exceeds the former dark value even more than the membrane potential does. For *Limulus* there are no data available in the same context. For an explanation of the blowfly and barnacle discrepancies between membrane resistance and membrane potential, the suggestion made by Koike, MackBrown and Hagiwara[5] of an electrogenic sodium pump which is stimulated by illumination seems a very valuable one.

Although the picture of photochemical conversions leading to electrophysiological events is far from complete as long as the particular nature of the excitors and inhibitors remains unknown, we think that our data support the supposition that a link can be demonstrated between the states of the photopigments (and their conversions into one another) and membrane phenomena. Meanwhile, the lack of a unique relationship between receptor potential and resistance changes might be held to lend support to the concept of an electrogenic sodium pump.

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Note: added in proof: But even the action of such a pump cannot account for our most recent observation, namely that at the onset of illumination the resistance reaches a new value in less than 5 ms and maintains that value even though the receptor potential falls in 50 ms to half its initial height. On this time scale no pumping mechanism can be held responsible for deviations from a unique relationship, which thus must be discarded.

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Axonal wiring and polarisation sensitivity in eye of the rock lobster

ARTHROPOD compound eyes consist typically of square or hexagonal facets beneath each of which lies a group of photo-receptor cells. Each photoreceptor or retinula cell gives rise to a microvillar fringe, called the rhabdomere, which consists of parallel tubules containing light-absorbing visual pigments. Axons arising from the retinula cells of each group are usually arranged in distinct bundles. These pass through the basement membrane and enter the first optic ganglion, the lamina, where they synapse with second-order neurones.

A modified organisation has been found only in Diptera[1] and now in Decapoda: in both these unrelated orders of arthropods, retinula cell axons interweave before they terminate on axons of the ganglion cells in the lamina cartridges. Whereas in Diptera this rather special arrangement is generally correlated with the 'open' type of rhabdom[2] (that is, each rhabdomere has its own visual axis and interweaving of retinula axons of several complete visual units, or ommatidia, results in a summation of like axes), the situation is different in rock lobsters, for they possess a 'fused' rhabdom characterised by centrally-connected rhabdomeres.

To determine the functional significance of the interweaving axons occurring in this type of compound eye, I traced the pathways of retinula cells and their axons in the eye of the rock lobster. Since Eguchi et al.[3] have identified the locations of colour-sensitive cells in the similar retina of the crayfish, and Hafner[4] has examined the fine-structural organisation of the lamina ganglionaris in the same animal, it also became necessary to know more about the region in between retina and lamina.

The rhabdom of *Panulirus longipes*, the Western Rock Lobster, is tiered and consists of seven large retinula cells. An eighth cell exists, but it is small and inconspicuous and apart from a tiny portion at the very distal end of the 'distal rhabdom' does not contribute to the rhabdom. As in *P. argus*[5] the distal rhabdom consists of alternate layers of perpendicularly-oriented microvilli belonging to the rhabdomeres of the seven contributing retinula cells. One set of alternative layers is made

Fig. 2 Serial sections (2 μm), stained with toluidine blue and examined under the light microscope, reveal how the regular interweaving pattern of retinula cell axons is produced. Erroneous combinations are extremely rare. Each retinula cell is approximately 15–18 μm in diameter.

up by the microvilli of three retinula cells, the other set, which has its tubules at right angles to those of the three cells, comprises microvilli of the remaining four cells. This type of rhabdom, commonly called 'banded' because of its appearance in longitudinal sections, seems to be typical of decapod crustaceans[5] and a few insects[6], and is thought to be significant for e-vector discrimination[7,8]. The proximal rhabdom in adult *P. longipes* (but not in larvae where it is also 'banded') differs from the thin cylindrical column of its distal counterpart in having a diameter which is ten times larger and microvilli which are not precisely oriented in two directions—as in the distal rhabdom—but run in all directions. In transverse section the proximal rhabdom resembles the leaf of a chestnut tree, in that there are seven large centrally-connected lobes.

The positions of the seven retinula cells are remarkably regular across the eye in each plane: in both left and right eye the seven retinula cells of each ommatidium are aligned diagonally so that four cells point anteriorly and three posteriorly (Fig. 1). The orientation of microvilli within these cells, however, may vary (Fig. 1a–d).

Serial sectioning revealed that some 50 μm above the basement membrane interommatidial bundles of retinula cell axons are formed (Fig. 2). The axons from seven retinula cells of one ommatidium interweave with those of their four neighbouring facets in such a way that four bundles of three fibres, heterogeneous with regard to their ommatidial origin, plus one single axon are produced (Fig. 3). The single axon consistently joins one particular bundle (Fig. 3), a few microns below the basement membrane. To find which of the seven retinula cells, in terms of microvilli orientation, became combined in these interommatidial bundles, transverse sections at the level of the distal rhabdom (that is, where the villi were precisely oriented in two orthogonal directions) were examined in the electron microscope.
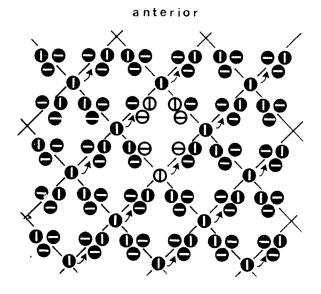
Once it was realised that the position of the narrow cone cell processes between the retinula cells was extremely consistent, identification of retinula cells became easy. The only cell with cone cell processes on both sides was called cell 1 and numbering of the remaining seven cells continued counter-clockwise.

In only very few cases was the arrangement of the seven cells similar to that reported by Eguchi and Waterman[5] for *P. argus* (Fig. 1a). Equally rare (that is, less than 5%), was the arrangement shown in Fig. 1b. Had either of these two arrangements been realised more frequently, a surprisingly simple connectivity pattern of interconnections would have resulted from the particular way that retinula cell axons interweave before they penetrated the basement membrane: axons of cells with the same microvilli orientation would have been brought together. The most commonly observed configurations

Fig. 1 Transverse section through the right compound eye of *P. longipes*. The seven retinula cells can be seen under a good binocular microscope at ×80. Axons of one ommatidium interweave with those of four neighbouring ones so that a cross of five 'naked' facets results. a–d, Schematic representation of retinula cell positions and microvilli orientations of two superimposed layers of the distal rhabdom. The position of the four crystalline cone processes, indicated by small black circles, provides an excellent reference for identification of retinula cells, for example, cell 1 always lies between two processes. With regard to the interweaving pattern described in this paper and illustrated in Figs 2 and 3 arrangements (a)—modified after Eguchi and Waterman[5]—and (b) would result in bundles in which all axons originated from retinula cells with parallel microvilli. Most commonly found, however, were arrangements (c) and (d), which are mirror images and which give rise to the interweaving pattern shown in Fig. 3.

were, however, retinula cells with microvilli arrangements as in Fig. 1c and d. The interweaving pattern of retinula cells in these cases would bring together the axons from cells which had their microvilli oriented at right angles to each other (Fig. 3).

There is evidence that the wiring pattern described is commonly found in decapod crustaceans with a 'banded rhabdom', but not in insects with a similar rhabdom[9]. For example, it has been reported[10] that interweaving occurred in the eye of *Astacus* and that groups of three or four axons, derived from neighbouring ommatidia, penetrated the basement membrane. For the European lobster *Homarus* retinula fibres were reported[11] to "separate and end in different optic cartridges", but there was no further investigation.

Little is known so far of the functional significance of the described axonal arrangement but Horridge[12], who has correctly predicted interactions between retinula cells of one ommatidium with those of four neighbouring facets, believes

anterior

posterior
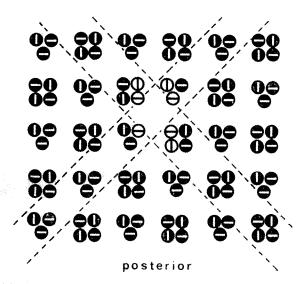
Fig. 3 Interweaving of retinula cell axons of one ommatidium (white circles) with those of four neighbouring facets results in four bundles of three fibres and one single axon (top). At 10 μm below the basement membrane (bottom) the single axon joins the anterior bundle to the right (= ventral in the right eye), thus producing an array which may be envisaged as two sets of bundles: each set being composed entirely of three or four axon bundles in rows. As symbolised by the bar in each circle, unlike the bundles containing three fibres, each bundle of four consists of equal numbers of axons originating from cells with microvilli oriented at right angles to each other.

that this could make movement perception independent of colour change or polarisation plane.

Although these findings do not support Muller[13], who believes that the frequently reported high polarisation sensitivity of individual retinula cells in crustaceans[7] is partly achieved by electrical coupling between cells which are sensitive to the same orientation of the e vector of polarised light, they do not automatically lead to the conclusion that polarisation sensitivity will be lost during transmission to the central nervous system. Even with our wiring pattern, connections which would ensure that some information on polarised light would reach the brain, are possible.

Alternatively, however, it could be argued that a two-channel–analyser, such as the banded rhabdom, is a convenient way to achieve a polarisation-independent system if receptor cell outputs are summed[14]. Because polarisation patterns are much more prominent in the sea than in air[15] (because of the dispersion of small particles in the water) it may be advantageous to a marine organism to be insensitive to them. The observed pattern of interweaving axons could be a direct consequence of this. The eighth retinula cell in the crab *Grapsus* with its microvilli arranged in two orthogonal directions[16], the relatively poor responses from adult crabs to polarised light in behavioural experiments[17], the failure to demonstrate an effect of polarised light on movement-sensitive fibres in the optic tracts of the crab *Podaphthalmus*[18] and the tendency for the axons of retinula cells with microvilli oriented at right angles to be brought together in *P. longipes*, certainly support the latter interpretation.

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Membrane proteins related to water transport in human erythrocytes

CLASSICAL interpretations of the mechanism of water transport across mammalian red cell membranes assume the existence of aqueous membrane pores[1-3]. As the permeability coefficient to water measured under an osmotic pressure gradient is usually significantly higher than the corresponding value measured under diffusional flow, the human red cell membrane is thought to act both as a selective solvent and a molecular sieve. Its ability to function as a molecular sieve depends on the existence of the pores, which could be assembled from aggregates of integral membrane proteins which span the membrane[4]. It is generally agreed that at least two proteins span the human red cell membrane[5-7]. Using polyacrylamide gel electrophoresis we have found that a band which contains one of these proteins is selectively labelled by a water-transport inhibitor.

We used 5,5′ dithio-*bis*-(2-nitrobenzoic acid) (DTNB), one of a group of sulphydryl compounds[8-10] that inhibit osmotic
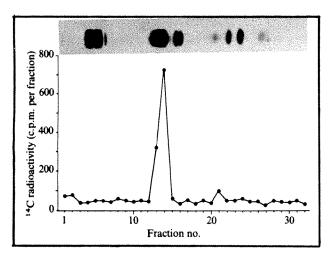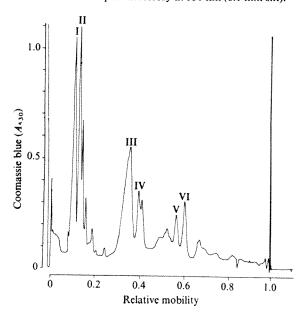
Fig. 1 Staining and labelling profiles of erythrocyte membrane proteins isolated from ^{14}C-DTNB-labelled cells. Ghosts isolated from red blood cells incubated with IAM, NEM and ^{14}C-DTNB were analysed for protein and radioactivity distribution by poly-acrylamide SDS gel electrophoresis. The gels contained 5.6% acrylamide and 1% SDS and were prepared and stained by the method of Fairbanks et al.[12]. Gels with radioactivity were sliced into 2-mm fractions, dissolved and analysed for distribution of ^{14}C-DTNB in a liquid scintillation counter. The amount of protein applied to each gel was 50 μg. Densitometry of gels is shown in Fig. 2. The identification of protein bands is according to the nomenclature of Fairbanks et al.[12].

water flow across some biological membranes. DTNB reacts with the smallest number of membrane thiol groups[11] (that is 10%, 2×10^{-17} mol per cell) of all the SH group reagents that inhibit water transport. Furthermore, it is specific for SH groups in that it only reacts by sulphydryl–disulphide exchange. To minimise nonspecific binding of DTNB to membrane proteins we preincubated cells with N-ethylmaleimide (NEM) plus iodoacetamide (IAM) which react with 60% and 30%, respectively, of the membrane SH groups[11]. These SH group reagents neither inhibit water permeability, nor do they affect the inhibitory action of DTNB[8]. NEM and IAM were also included in the cell lysing solution, as well as during the washing of the erythrocyte ghosts, so as to react with released SH-containing compounds which potentially could remove membrane-bound ^{14}C-DTNB.

Fig. 2 Densitometry of Coomassie blue stained gel in Fig. 1. Gels were scanned in a Gilford spectrophotometer with a model 2410 linear transport accessory at 530 nm (0.1 mm slit).

Fresh blood (25 ml) was mixed with 1 ml of 0.2M EDTA and an equal volume of cold 5 mM sodium phosphate–0.15 M NaCl (pH 7.4) and centrifuged at 1,000g for 10 min at 4 °C. Supernatant and buffy coat were removed and the packed cells were washed once and resuspended (30% haematocrit) in the buffered salt solution containing 1 mmol each of IAM and NEM. After incubation for 30 min at 37 °C, ^{14}C-DTNB (0.3 μmol) was added for a further hour, which is the time required for maximal inhibition of water transport. The cells were then collected by centrifugation and washed twice with the same incubation medium but without DTNB. Cell membranes were obtained by lysing 1.0–1.2-ml samples of the cell suspension in 40 ml of ice-cold 5 mM sodium phosphate solution, pH 8.0, containing 1 mM IAM and NEM. After centrifugation (20,000g for 30 min) the supernatant, which contained little radioactivity, was removed by aspiration. The ghost pellet was then obtained free from contaminating proteases in the tightly packed button, as described by Fairbanks et al.[12]. Ghosts were washed three times in this way and finally dissolved by addition of an equal volume of a solution containing 2% sodium dodecyl sulphate (SDS),
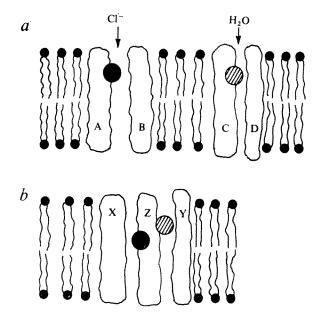
Fig. 3 a, Water and anion channels may be composed of entirely distinct polypeptide chains (although A may = B and C may = D) so that DIDS (filled circle) and DTNB (hatched circle) bind to separate polypeptide chains. b, Anion and water channels may be composed of several polypeptide chains, one of which is a common structural component of both (Z). DIDS and DTNB could react at different sites on Z thereby interfering with anion or water transport selectively.

20 mM Tris-HCl (pH 7.4) and 2mM EDTA. Aliquots of the dissolved membrane solution were then subjected to gel electrophoresis until the tracking dye ran 84 mm. The gels were stained with Coomassie blue, as described by Fairbanks et al.[12]. Duplicate gels, unstained, were sliced and dissolved for liquid scintillation counting of ^{14}C. The gels were calibrated with chymotrypsinogen (25,000 daltons), ovalbumin (45,000 daltons) and γ-globulin (160,000 daltons) as molecular weight markers. The gel staining pattern and the distribution of radioactivity are shown in Fig. 1. Only one Coomasie blue-staining band was labelled with ^{14}C-DTNB. This was band III which comprised about 30% of the total membrane protein[12], and had a molecular weight of about 95,000 (ref. 13). Labelling was not observed if NEM and IAM were omitted during lysis or if dithioerythaitol was added to lysed ghosts.

Gel densitometry was done using a Gilford spectrophotometer and model 2410 linear transport accessory, scanning at 530 nm (0.1 mm slit). The gel scans (Fig. 2) are essentially the same as those of Fairbanks et al.[12] except for the better resolution of the

two bands on the downward shoulder of peak II, and the resolution of peak IV into two bands. The latter was also observed by Fairbanks et al.[12] at lower concentrations of SDS. Figures 1 and 2 show that band III is quite dispersed and it may comprise more than one species of polypeptide[14]. This band has been reported to contain a phosphorylated intermediate of the (Na^+-K^+)-ATPase[15,16]. It also binds 4,4'-diisothiocyano-2,2-ditriostilbene-disulphonate (DIDS), a specific inhibitor of anion permeability.[13] The heterogeneity of protein band III is also indicated by the observation of Cabantchik and Rothstein[14] that after Pronase treatment the staining of this band decreased and three individual bands were revealed. Based on studies involving proteolysis with chymotrypsin or pronase, Cabantchik and Rothstein[14] concluded that ^{3}H-DIDS was bound to a hydrophobic 65,000-dalton fragment of the 95,000-dalton protein.

Thus the labelling of band III by DIDS, an anion permeability inhibitor, and DTNB, a water permeability inhibitor, may result because each agent binds specifically to a different polypeptide chain. Another possibility is that the anion and water channels are each formed from a common structural polypeptide chain plus one or more polypeptide chains specific for the particular functional requirement (Fig. 3). DIDS and DTNB could conceivably react at different sites on the common structural polypeptide chain thereby impeding the function of only one type of channel in each case. We conclude from these experiments that polypeptide chains of molecular weight about 100,000 form part of the structure of aqueous channels which regulate non-carrier mediated transport in human erythrocyte membranes.

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Abnormalities in membrane microviscosity and ion transport in genetic muscular dystrophy

WE have demonstrated recently several functional differences between membrane-bound enzymes from tissues of muscular dystrophic chicks and of controls[1]. Several other membrane anomalies have been associated with this disease[2-7], but so far the only known biochemical anomaly in the affected membranes is a difference in lipid composition[8,9]. This could cause all the observed functional defects, including those in enzyme activities and permeability, through an alteration in the membrane microenvironment[10]. Certain physical properties of the microenvironment can be studied by fluorescent polarisation techniques that measure the mobility of a lipid-soluble fluorescent dye[11,12]. The basis for this approach is the fact that

membrane cholesterol, which strongly affects the microviscosity of liposomes[10], is present in significantly greater than normal amounts in genetic muscular dystrophy. Using this approach, we found (Table 1) that the membranes of muscle, liver and erythrocytes of dystrophic chicks have a significantly higher microviscosity than normal controls. The greatest difference was in muscle, the site of the clinical symptoms of this genetic disease. The higher cholesterol:phospholipid ratio characteristic of the diseased membrane is sufficient to account for the observed increase in microviscosity[11].

Probably the most significant pathological expression of the defect of muscular dystrophy is an alteration in ion transport. It has been demonstrated that potassium efflux from dystrophic mouse muscle and human erythrocytes is significantly greater than normal[13,14]. In view of the potential use of these observations in the screening and diagnosis of Duchenne muscular dystrophy (DMD), and in order to gain a deeper understanding of the defect we investigated the potassium and sodium transport properties of human erythrocytes. The results (Table 2) show that the potassium influx is significantly greater in both patients and carriers of the disease, relative to control. Ouabain, which inhibits the active component of the Na^+ and K^+ ion fluxes and the closely coupled ATPase activity[15], inhibited (at 10^{-4} M) the potassium influx and the sodium efflux in the erythrocytes of all three groups. Thus the erythrocytes of DMD patients and carriers have a functional cation pump. On the other hand, Brown et al. showed that in the presence of Na^+ and K^+ ouabain enhanced the ATPase activity of erythrocytes from DMD patients (while it inhibited the ATPase activity of matched controls)[16]. The patients with DMD represent the first example of dissociation of the ouabain-inhibited component of sodium efflux and potassium influx from the ATPase activity. Together with the observation of the increased potassium efflux, this finding points to an imbalance between the active (pump) and passive (leak) ion movements in this disease.

The following tentative explanation relates the abnormality in ion fluxes to the observed increase in lipid microviscosity. The higher cholesterol: phospholipid ratio causes a tighter packing of lipid molecules resulting in an enhancement of the Van der Waals' interaction between adjacent lipid molecules[17]. These changes may create a defect in the polar pathways in the protein moiety of the membrane. This would increase the passive ion movement and cause an imbalance between the

Table 1 Membrane microviscosity in muscle sarcolemma, liver and erythrocyte plasma membranes of normal and dystrophic chicks

Tissue	Membrane microviscosity, $\bar{\eta}$, (cP)
Normal muscle	61 ± 8 (7)
Dystrophic muscle	117 ± 7 (14)*
Normal erythrocytes	365 ± 30 (6)
Dystrophic erythrocytes	435 ± 20 (14)*
Normal liver	153 ± 15 (14)
Dystrophic liver	234 ± 15 (19)*

*Significantly different from control at $P < 0.01$.

Muscle sarcolemma, liver and erythrocyte plasma membranes were prepared as before[1,18-20] from the respective tissues of 20-d-old dystrophic chicks and age matched white leghorn (No. 25 Arbor Acre) control chicks. Steady-state fluorescence polarisation was measured with a Hitachi MPF-2A fluorescence spectrophotometer. Perylene at 10^{-6} M was used as the fluorescent probe. The dye was excited at 436 nm and emission was measured at 474 nm. Anisotropy of fluorescence was measured by polarising the exciting beam with a Polacoat ID5UV filter and observing the emission through the same type of filter. Fluorescence lifetimes were measured with a calibrated Ortec 9200-ns spectrometer. The calculation of the membrane microviscosity, $\bar{\eta}$, was based on the following relationship[11] $r_0/r = (1 + KT\tau)/\bar{\eta}V(r)$ where r_0/r, is the anisotropy τ is the mean fluorescence lifetime, K is Boltzman's constant, T is absolute temperature and $V(r)$ is the effective rotational molecular volume. Results represent the mean $\pm$ s.d. The number of determinations is given in parentheses.

Table 2 Potassium influx and sodium efflux in erythrocytes of DMD patients, carriers and controls (meq l^{-1} erythrocytes × h)

Source of erythrocytes	Potassium influx		Sodium efflux	
	Total	+Ouabain	Total	+Ouabain
Normal subjects	1.90 ± 0.05 (3)	0.35 ± 0.03 (3)	3.6 ± 0.5 (2)	1.4 ± 0.3 (2)
Duchenne dystrophy patients	2.20 ± 0.06 (4)*	0.43 ± 0.03 (4)	3.5 ± 0.4 (4)	0.9 ± 0.1 (4)
Genetic carrier patients	2.25 ± 0.10 (4)*	0.50 ± 0.04 (4)		

*Significantly different from control at $P < 0.01$.
Blood was collected in heparinised syringes, centrifuged for 15 min at 1,500*g*. The plasma and buffy coat were removed carefully and the cells were washed three times in buffer as before[21]. Flux measurements were carried out following standard techniques[21,22]. Results represent the mean and s.d. The number of determinations is given in parentheses.

active and passive ion transport. This study thus further supports the view that a lipid-related defect which affects the membrane microenvironment is the underlying genetic disorder of muscular dystrophy. The localisation of the pathological manifestation of the disease in the muscle is probably a reflection of the higher susceptibility of its specialised membrane system to this defect.

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Association of actin and myosin with secretory granule membranes

ACTIN and myosin have been found in many non-muscle cells[1]. They are presumed to function in the generation of the force required in the various expressions of cytoplasmic or cell motility. Although both have been found in association with the plasma membrane[2-5], nothing is known about the nature or consequences of this association. Equally uncertain is the mechanism of movement of secretory granules : synaptic vesicles, for example, are transported along neurones at high rates (up to 20 cm d^{-1})[6], probably in association with microtubules[7]. Roles for actomyosin in this process[8] and in exocytosis[9] have been proposed. We have used a new approach to the problem of actomyosin-membrane interaction, and report here the binding of actin

and myosin *in vitro* to a purified cytoplasmic membrane component.

Chromaffin granules, the catecholamine-containing vesicles of the adrenal medulla, are homologous in origin to the synaptic vesicles of sympathetic nerve cells. Both contain antigenically identical storage proteins[10] and similar membrane enzymes[11]. Bovine chromaffin granules are easily purified[12] and their membranes are released by lysis. We modified the conventional preparative methods by isolating the granules in 0.15 M KCl, buffered with 10 mM HEPES pH 7.0, and purifying them by centrifugation (40 min at 4 °C and 150,000*g* through 1.5 M sucrose in the same medium. Suspension of the pellet in buffered 0.15 M KCl leads to extensive lysis of the granules. The resulting mixture of membranes and partially-lysed granules was collected by centrifugation and used in subsequent experiments at a concentration of about 6 mg protein per ml. This membrane preparation resulted in some differences in minor protein bands on SDS polyacrylamide gel electrophoresis when compared with membranes prepared at low ionic strength[12].

The adrenal medulla contains both actin and myosin (ref. 13 and K. B. and J. H. P., unpublished). We have investigated the binding of smooth muscle myosin from chicken[14] and of rabbit actin to chromaffin-granule membranes using the following method. Partially-lysed granules were incubated with the proteins under study (generally in 0.25 ml of 0.15 M KCl containing 10 mM HEPES and 1 mM $MgCl_2$ for 30 min at 24 °C); the suspension was then mixed with 0.5 ml 70% (w/w) sucrose in the same medium in a centrifuge tube (Spinco 2 × 0.5 inch), and overlaid with a linear gradient of sucrose (1.6 M to 0.6 M in the medium used for incubation). During the subsequent centrifugation (3 h at 4 °C and 200,000*g*), free proteins (storage proteins that have leaked from the damaged granules, together with test proteins that remain unbound) remain at the bottom of the tube, together with any unlysed granules; free membranes float up the tube to form a band at a position corresponding to their hydrated density (rather a broad band with a mean density of about 1.15 g ml^{-1} for membranes prepared by this method). Ten fractions were collected from each gradient and the proteins in each fraction were analysed by electrophoresis in polyacrylamide slab gels containing sodium dodecyl sulphate[15], followed by staining with Coomassie brilliant blue.

An analysis of granules incubated in the absence of other proteins is shown in Fig 1*a*. The major bands are chromogranins, the storage proteins found inside the granules. They are found in high concentration at the bottom of the gradient (fractions 1–3); the membranes, which are not obtained free of chromogranins, form a band in fractions 6–8. In some experiments the partially-lysed granules were subjected to freezing and thawing (Fig. 1*c* and *d*); in this case the membrane band is found further up the gradient, corresponding to a lower density.

If F-actin is incubated with the granules subsequent analysis of the gradient on SDS gels (Fig. 1*b*) shows that actin is distributed throughout the gradient; it is now a sig-
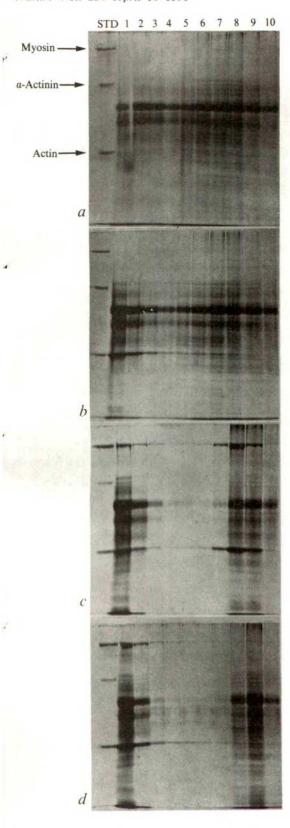
Fig. 1 SDS polyacrylamide slab gel electrophoresis showing fractions of sucrose gradients of chromaffin-granule 'ghost' membranes after incubation with test proteins. *a*, Membranes only; *b*, membranes and rabbit F-actin; *c*, membranes and F-actin and myosin (from chicken smooth muscle); *d*, as (*c*) but with 5 mM Mg-ATP during incubation and in the gradient. The left-hand column (STD) of each gel contained standard protein markers, myosin, α-actinin and actin; 1 represents the bottom fraction and 10 the top fraction of each gradient. Every fraction of a gradient is shown. The membranes in (*c*) and (*d*) had been frozen and thawed once and consequently having a lower density band higher up the gradients. Sucrose gradients were run as described in the text.

nificant component of the membrane fractions. This distribution is not affected by increasing the centrifugation time to 14 h. If ghosts prepared by the conventional low ionic strength procedure are used little actin is taken up the gradient. If F-actin is incubated in the absence of granule membranes it remains at the bottom of the gradient (no trace is found above fraction 3).

A very small amount of myosin can be shown to bind in the same way when myosin is incubated with membranes in the absence of actin; this binding is not affected by the method of preparation of the membranes. If, however, both actin and myosin are included in the incubation simultaneously, there is a great enhancement of the binding of each (Fig. 1*c*), presumably because of actin–myosin interaction on the membranes. This effect is abolished by the addition of excess (5 mM) Mg-ATP; inclusion of this in the incubation and the gradient reduces the binding of both proteins to the levels found in the absence of each other (Fig. 1*d*). These background levels of binding do not seem to be sensitive to Mg-ATP or to 2 mM Mg-pyrophosphate. Two out of twelve preparations of granules tested failed to bind actin to the extent shown in Fig. 1*b* although they continued to bind myosin; preliminary experiments suggest that this is caused by proteolytic action. Such preparations fail to show the enhancement effect with actin and myosin, suggesting that this enhancement depends on the successful binding of actin to the membranes.

We tried to assess the specificity of actin and myosin binding by incubating the membranes with other purified proteins and with a post-microsomal supernatant fraction from the adrenal medulla. In the first case, β-galactosidase, catalase and pyruvate kinase failed to bind, although a trace of phosphorylase A and tropomyosin binding was detected. Of the total supernatant proteins only three bound significantly, their mobilities on the gel corresponding to molecular weights of approximately 90,000, 70,000 and 45,000, the last probably being actin.

Fractions from the sucrose gradients were examined using electron microscopy. Filaments were frequently found to be associated with chromaffin-granule membranes when material from the main band of a gradient such as that shown in Fig. 1*b* was examined (Fig. 2); control gradients (Fig. 1*a*) contained no filaments. Many gradients were examined: a striking finding was that filaments generally terminated in a membrane (Fig. 2) and were rarely found to cross membranes (Table 1). Attachment of membranes to the centres of filaments might have been expected if membrane-bound myosin was involved.

The number of membrane 'ghosts' bearing filaments varies greatly from one experiment to another. The maximum

Table 1 Association of chromaffin-granule 'ghosts' with actin filaments

Experiment	Number of filaments counted			
	First	Second	Third	Total
Unassociated	113	34	86	233
Centrally-associated	26	11	24	61
Terminally-associated	99	30	48	177
Terminally-associated, but crossing an additional 'ghost'	14	1	2	17
Total number of filaments counted				488

Each experiment represents a different batch of chromaffin granules. Grids (see Fig. 2) were scanned at a magnification of 10,000 or 20,000, higher magnifications being used if necessary. Every filament found in a grid square was counted unless it could not be assigned unambiguously (for example, filaments crossing grid bars). 'Association' refers to apparent contact between a filament and an intact or fragmented membrane 'ghost' (see Fig. 2). Filaments which completely crossed, or which were tangential to membrane 'ghosts' were counted as 'centrally-associated'. The number of terminally-associated filaments which cross an additional 'ghost' is dependent on the frequency of 'ghosts' on the grid; this suggests that many 'centrally-associated' filaments are filaments with membranes inadvertently lying over them.
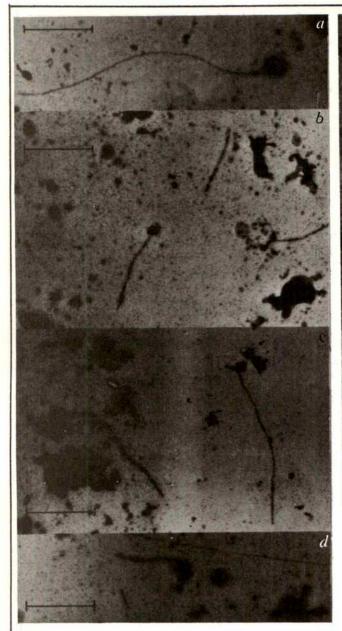
Fig. 2 Electron micrographs of chromaffin granule membranes with associated actin filaments. After incubation with F-actin the membranes were purified from unattached protein by sucrose gradient centrifugation. Material from the peak membrane fractions was placed on carbon grids, 'decorated' with myosin subfragment 1 (ref. 16) and stained with 1% uranyl acetate. *a*, *b*, *c* and *d*, Low power views of typical grids (the bar represents 2 μm). Some of the membranes show associated actin filaments. Free filaments are also visible. *e*, *f* and *g*, Membrane–F-actin terminal associations at higher magnification (bar represents 0.5 μm). In (*e*) the arrowhead polarity of the myosin S_1 is towards the membrane, and in (*f*) and (*g*) the polarity is away from the membrane.

number found has been about 10% per preparation. All preparations contained unattached filaments, however, even though specimens were taken from the main band on the gradient; such filaments were often short and may have arisen by fragmentation on the grid.

The attached actin filaments had both polarities as judged by their 'arrowhead' directions : 46 out of 68 examined had 'arrowheads' pointing away from the membrane. ('Decoration' of a filament attached to a muscle Z line shows 'arrowheads' pointing uniformly away from the attachment point[16].) We did not find a convincing case of more than one filament attached to a 'ghost', although aggregates of 'ghosts' with several emerging filaments were sometimes observed.

Our experiments demonstrate that myosin and filamentous actin interact independently with secretory granule membranes. Because of the complexity of the present assay it has been difficult to investigate the specificity of these interactions and their optimal conditions, and we do not yet know whether protein–protein interactions are involved. In view of the high frequency of interaction of membranes with the ends of actin filaments, it is attractive to speculate that there may be an actin-binding site in these membranes. Our finding that 'ghosts' prepared conventionally at low ionic strength bind very little actin is consistent with such a binding site being an α-actinin-like protein[17], which is extracted at low ionic strength. Such a suggestion becomes more plausible now that one of us has identified a protein similar to α-actinin, in molecular weight and actin binding properties, from the particulate fractions of brain and fibroblasts (K. B., unpublished). If further work confirms a binding site, then the attachment of actin to these membranes could have a role in the transport of secretory granules from their site of formation in the Golgi apparatus to sites of exocytosis at the plasma membrane.

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Thiamine increases the specific activity of human liver branched chain α-ketoacid dehydrogenase

THIAMINE pyrophosphate (TPP), the active derivative of vitamin B_1, functions as a coenzyme in dehydrogenase reactions such as the oxidative decarboxylation of α-ketoisovaleric (KIV), α-keto-β-methylvaleric (KMV) and α-ketoisocaproic (KIC) acids in humans[1] Impairment of these reactions produces the group of disorders known as maple syrup urine disease[2,3] In the classic form of the disease, the ketoacids and their amino acid precursors accumulate in homozygous affected individuals and depress the function of the central nervous system early in life Therapy involves restriction of branched chain amino acids in the diet to decrease their concentrations in the body fluids[4] Further reduction in plasma leucine, isoleucine and valine has been achieved by supplementing this diet with thiamine for patients with partial (60%) and severe (95%) reduction in enzyme activity[5,6] Other investigators found no effect in patients lacking all enzyme activity[7,8] We are seeking an explanation for the clinical response at the enzyme level and have already reported that branched chain α-ketoacid dehydrogenase activity was increased in peripheral white blood cells This occurred after 3 weeks of oral thiamine treatment in patients with 5% activity and in normal controls[5] In contrast, activity in mitochondrial inner membranes from cultured normal and mutant skin fibroblasts was not stimulated directly by TPP/Mg^{2+}, but was prolonged by the presence of TPP/Mg^{2+}, suggesting that the cofactor increases the half life of the dehydrogenase complex We have now found that thiamine supplementation of normal diets increases normal human liver branched chain α-ketoacid dehydrogenase activity

Mitochondrial inner membranes were isolated from human liver obtained by open biopsy, and branched chain α-ketoacid dehydrogenase was measured before and during *in vivo* administration of 100 mg thiamine per day Tissue was kept in ice cold 0 27 M mannitol buffered with 1 0 mM Tris-HCl, pH 7 3, and 0 1 mM EDTA (MTE) Tissues were assayed within 30 min after samples were taken A decrease in mitochondrial dehydrogenase activity was noted if tissues were stored, cold or frozen After homogenisation with a glass–Teflon tissue grinder, mitochondria were isolated by differential centrifugation and washed once with MTE Mitochondria

Table 1 Effect of oral thiamine on normal adult human hepatic branched chain α-ketoacid dehydrogenase

Substrate	Duration of 100 mg d⁻¹ oral thiamine		
	0 d (6)	5–15 d (9)	18–28 d (3)
	(nmol CO_2 per 15 min per mg protein)		
KIV	18 92±5 34	19 84±3 90	35 29±6 96
KMV	9 53±2 15	8 38±2 24	12 79±2 65
KIC	8 25±2 73	6 88±1 24	14 89±2 61

$P < 0 001$

Assay conditions for CO_2 evolution are as follows in 0 25 ml cofactors were present at a final concentration of 0 2 mM TPP, 0 3 mM $MgCl_2$, 0 2 mM CoA and 0 2 mM NAD^+ in 50 mM Tris-HCl, pH 7 4 and 0 2 mM EDTA, and substrate at 2 mM Mitochondrial protein was between 60 and 600 µg $^{14}CO_2$ evolved, was trapped and quantitated as previously described[1] The number of patients in each time group is shown in parentheses Values represent the mean and standard deviation of at least quadruplicate observations *P* values were obtained using Student's *t* test and represent the probable difference between conditions after 5–15 d and 18–28 d for all three substrates

were then suspended in MTE and mixed with an equal volume of MTE containing digitonin so that the final concentration was 1 mg digitonin per mg mitochondrial protein Detergent action was stopped by addition of an equal volume of MTE after the reaction had proceeded for 20 min at 0 °C Inner membranes were isolated immediately by centrifugation at 10,000g for 15 min and then suspended in an appropriate amount of MTE for use in the experiment

Two methods of assay were used to follow dehydrogenase complex activity according to the products formed in the overall reaction

$$R\text{-}C\text{-}*C\text{-}O^- + CoASH + NAD^+ \xrightarrow{TPP/Mg^{2+}} R\text{-}C\text{-}SCoA + *CO_2 + NADH$$

Using 1-^{14}C-labelled ketoacids as substrate, $^{14}CO_2$ produced was trapped in hyamine for quantitation by liquid scintillation This assay was sensitive at pmol quantities of evolved product The concentration of NADH was determined by spectrophotometry using an $\varepsilon = 6\ 22 \times 10^3$ Mcm⁻¹ at 340 nm under assay conditions as described in Table 1 Optical clarity was required for this assay One mole of CO_2 was released for every mole of NAD^+ reduced In a typical experiment with 2 mM KIV as substrate, 13 82 nmol CO_2 per 15 min per mg were produced while the rate of NADH formation was 13 65 nmol per 15 min per mg

Thiamine was administered orally (100 mg d⁻¹) to individuals with presumed normal branched chain dehydrogenase activity for up to 28 d before surgery, when open liver biopsy was performed for other diagnostic purposes The dehydrogenase activity of mitochondrial inner membranes from these samples was measured and compared with activity in untreated controls

Thiamine did not influence the branched chain α-ketoacid dehydrogenase activity until supraphysiological loading had been present for at least 18 d (Table 1) After this time, a 1 5–2 0-fold increase in specific activity was observed towards all three substrates

There are several explanations for these results First, thiamine could increase synthesis of these mitochondrial proteins Second, thiamine or TPP could activate preformed inactive components of this complex This is unlikely since time was required for the *in vivo* effect and *in vitro* addition of TPP to the assay media did not increase the specific activity A third possibility is that thiamine or TPP at these supraphysiological concentrations maintains a conformation of the multienzyme complex which is less susceptible to degradation than is the naturally occurring holoenzyme Such a mechanism has been postulated to explain the increase in hepatic pyridoxal-

5′-phosphate-dependent cystathionine synthase activity after prolonged administration of pyridoxine[9]

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Fig. 1 Concentration dependency of ppGpp inhibition (results of experiments shown in Table 1) The percentage rRNA of total RNA has been calculated from the percentage ^{3}H-UMP in rRNA by multiplication with 1 25 to correct for the base composition of rRNA relative to total DNA–complementary RNA O, Total RNA, ●, percentage rRNA, ▲, total rRNA

Specific inhibition of ribosomal RNA synthesis *in vitro* by guanosine 3′ diphosphate, 5′ diphosphate

CONSIDERABLE evidence has accumulated that guanosine 3′-diphosphate, 5′-diphosphate (ppGpp)[1] is involved in the regulation of ribosomal RNA (rRNA) synthesis in *Escherichia coli in vivo* (see ref 2) The involvement of ppGpp is most convincingly indicated by results obtained under conditions of amino acid starvation A strong increase in ppGpp accumulation is always accompanied by a concomitant decrease in stable RNA accumulation[3] *In vitro*, a specific effect of ppGpp has not been found, neither in a purified[4], nor in a crude system[5] Travers *et al*[6,7] have proposed that ppGpp specifically affects rRNA synthesis by counteracting the effects of the protein elongation factor EF-Tu Here, we show that ppGpp can specifically inhibit rRNA synthesis *in vitro* using purified RNA polymerase with two templates—DNA prepared by phenol extraction, and nucleoids prepared using methods developed by Pettijohn *et al*[8]

Table 1 shows that our hybridisation competition system has a very high specificity for rRNA, a low background and a high hybridisation efficiency These characteristics together make it possible to detect clearly even small differences in rRNA synthesis

Using nucleoids as template we found that 3 9±0 3% (s d

mean) rRNA was made by added, purified RNA polymerase Reaction conditions were as described in Table 1, except for the about tenfold less DNA concentration ppGpp (0 6 mM) decreased total RNA synthesis by about 20% whereas rRNA was on the average 45% lower, resulting in a mean percentage rRNA synthesis of 2 7±0 4% In four different experiments, the percentage rRNA was always lower when ppGpp was added

The effect of ppGpp on total and rRNA synthesis was also assayed at various RNA polymerase–DNA ratios using phenol-extracted DNA At all ratios tested ppGpp specifically inhibited rRNA synthesis (Table 2), the inhibition seems to be less pronounced at high and low ratios The concentration dependency of ppGpp inhibition is shown in Fig 1 Both total and ribosomal RNA synthesis are progressively inhibited by higher concentrations of ppGpp Half maximal inhibition is obtained at about 0 15 mM which is the same as the apparent Ki for stable RNA accumulation *in vivo*[12] GDP (1 mM) did not decrease total or rRNA synthesis

As we found in all conditions studied a specific effect of ppGpp on rRNA synthesis, that is, a stronger inhibition than that of total RNA synthesis, it is remarkable that others did not find this effect Our hybridisation method is very sensitive and will allow detection of small differences, even at low rates of rRNA synthesis In our case, inhibition of total RNA synthesis by ppGpp was less than that found by others[4,5] If inhibition of total RNA synthesis is large it could have 'drowned' a differential

Table 1 Hybridisation competition analysis of [^{3}H] -RNA synthesised in vitro

ppGpp conc (mM)	Input ^{3}H-UMP (d p m) in RNA	^{3}H-UMP (d p m) in hybrid −rRNA	^{3}H-UMP (d p m) in hybrid +rRNA	Competed	^{32}P-rRNA (d p m) in hybrid −rRNA	^{32}P-rRNA (d p m) in hybrid +rRNA	^{3}H-UMP (d p m) in rRNA	^{3}H-UMP in rRNA (%)
0 0	321,595	16,107	4,031	12,076	2,848	176	18,069	5 6
0 14	294,338	11,031	3,193	7,838	2,781	150	11,888	4 1
0 28	281,434	8,997	3,001	5,996	2,861	125	8,748	3 2
0 69	250,021	7,576	2,808	4,768	2,846	112	6,985	3,0
1 38	230,846	6,943	3,236	3,707	2,771	93	5,531	2 5

An aliquot (50 μl) of *E coli* DNA (44 μg ml⁻¹) was added to 0 45 ml of a reaction mixture containing 40 mM Tris-HCl, pH 7 9 (25 °C), 0 4 mM potassium phosphate, 10 mM MgCl₂, 100 mM KCl, 0 3 mM each of ATP, CTP and GTP, 0 1 mM EDTA, 0 1 mM dithiothreitol and 0 07 mM ^{3}H-UTP 1 Ci mmol⁻¹ The solution was incubated at 38 °C for 10 min, and then 32 μg RNA polymerase was added ppGpp was added just before RNA polymerase After 30 min at 38 °C one-quarter volume of 1 5 M NaCl containing 0 15 M sodium citrate (pH 7) was added, and the solution was extracted once with the phenol mixture of Kirby[9] Total RNA synthesis was measured in a 10 μl sample to which 100 μg yeast RNA was added as carrier by precipitation with 6% TCA containing 10 mM pyrophosphate The remainder of the water layer was used directly in the hybridisation test Hybridisation competition analysis was essentially according to Haseltine (ref 4, Fig 6), except that we used *Proteus vulgaris* DNA enriched for ribosomal DNA on a methylated albumin kieselguhr column instead of *E coli* DNA Values are the average of two triplicate hybridisations in which 20 μl *in vitro* ^{3}H-RNA and 4,005 d p m ^{32}P-rRNA were used ppGpp was prepared by the method of Cashel[10] Further details of the experimental procedures were as described[11]

Table 2 Effect of ppGpp at various RNA polymerase to DNA ratios

Ratio of RNA polymerase to DNA (w/w)	Percentage rRNA		Percentage inhibition	
	−ppGpp	+ppGpp	total RNA	rRNA
1 7	3 4	2 4	7	34
14	8 5	4 4	16	56
28	5 5	2 5	12	60
43	4 3	2 4	16	53
57	2 4	1 5	12	45

The experiments were carried out as described in the legend to Table 1 The final concentration of DNA was 15 μg ml^{-1} at a ratio of 1 7, and 4 4 μg ml^{-1} at the other ratios ppGpp was added at a final concentration of 0 6 mM

effect on rRNA Why inhibition of total RNA synthesis is lower in our system is unclear, our ppGpp preparation was checked for purity using polyethyleneimine thin layer chromatography (more than 90% pure)

The question arises how ppGpp effects its specific inhibition of rRNA synthesis We have found that with the nucleoids packing of added RNA polymerase molecules on the ribosomal cistrons is maximal[11] Consequently, the elongation rate will determine the initiation frequency and it cannot be decided whether ppGpp acts specifically on elongation or initiation of rRNA Further experiments are needed to clarify this point

Whatever the mechanism, our results clearly show that ppGpp can specifically inhibit rRNA synthesis by purified RNA polymerase on a DNA template without the addition of other macromolecular components Although extrapolation to the *in vivo* situation must be carried out with great caution our data support the hypothesis of a direct involvement of ppGpp in the (negative) control of rRNA synthesis

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Enhancement of interferon production by poly(rI)·poly(rC) in mouse cell cultures by ascorbic acid

We have reported[1] an increased response to interferon induction in mice fed a diet containing vitamin C, and have now found a similar phenomenon *in vitro* Addition of L-ascorbate to cultures of mouse cells (transformed L cells and normal embryonic fibroblasts) stimulated with polyinosinic acid polycytidylic acid (poly(rI) poly(rC)) resulted in increased synthesis of interferon

Mouse L cells (American Type Culture Collection, Rockville) were seeded in 2-oz prescription bottles (Brockway Glass Co , Brockway, Pennsylvania) at a concentration of 4×10^5 cells ml^{-1} in 25 mM HEPES-buffered Medium 199 (ref 2) with 10% decomplemented foetal calf serum Ascorbate was added to a final concentration of 10^{-4} M or 10^{-5} M and the cultures were incubated for 18 h at 37 °C A dose of 10^{-3} M ascorbate was also used initially, but it usually made cultures acidic, with subsequent toxic effects The medium was decanted, and the cell monolayer was exposed to synthetic poly(rI) poly(rC) (25 μg ml^{-1}, Miles) and diethylaminoethyl-dextran (100 μg ml^{-1}) for 1 h The cultures were washed three times with phosphate-buffered saline, refed with HEPES medium with included ascorbate and incubated at 37 °C for another 10 h The medium was then assayed by a colorimetric method[3] to quantify cytopathic effects in L cell monolayers with vesicular stomatitis virus (VSV) as the challenge virus Volumes of 1 ml of serial dilutions were used for assay, and the interferon titre, expressed in dye-uptake units (DU$_{50}$ ml^{-1}), was the reciprocal of the dilution which gave an absorbance reading at 540 nm midway between that of uninfected cell controls (100%) and the virus-infected controls (0%) Readings of duplicate dilutions generally agreed to within 10% A standard reference interferon preparation (NIH reference interferon G002-904-511) with an assigned activity of 12,000 U ml^{-1} was included with each assay, and these reference titres are presented along with the experimental results The NIH mouse reference interferon titres 22,500 U ml^{-1} as an average in repeated assays in our laboratory Since the assigned

Table 1 Effect of ascorbate on interferon induction by poly(rI) poly(rC) in mouse L cell cultures

Concentration of ascorbate (M)	Interferon titres*		
	Experiment 1	Experiment 2	Experiment 3
None added	1,450	1,100	1,665
$1 0 \times 10^{-5}$	1,785	ND	1,910
$1 0 \times 10^{-4}$	3,330	3,105	5,060
NIH reference	20,185	15,300	28,545

*Titres are expressed in DU$_{50}$ ml^{-1}
ND, not done

potency of the reference interferon is 12,000 U ml^{-1}, conversion to reference units can be made by dividing the experimental values in the tables by the factor 1 9 The viral inhibitor was inactivated by trypsin, was non-toxic to L cells, did not directly inactivate VSV, and showed no antiviral activity in normal chick embryo fibroblasts

The presence of ascorbate in cultures of L cells resulted in increased interferon production as demonstrated in three separate experiments Values for 10^{-5} M and 10^{-4} M ascorbate (Table 1) show a dose response effect Control cultures (no ascorbate added) produced a mean interferon titre of $1,405 \pm 285$ All three observations made with 10^{-4} M ascorbate exceeded this control value by more than three standard deviations and hence are considered significant

It is well known that cells maintained in culture lose their resemblance to normal cells In this connection, we have observed that primary and secondary cultures of mouse embryo fibroblasts are considerably less responsive to interferon induction by poly(rI) poly(rC) than are cells of the mouse L line Therefore, it seemed important to study the effects of ascorbate on cells newly established in culture The results of two experiments (Table 2) with secondary cultures of normal mouse embryo fibroblasts revealed that, as in the case of the L cell line, 10^{-4} M ascorbate substantially increased the production of interferon

Statistically, the interferon titres observed with 10^{-4} M ascorbate in both cases exceeded the mean value of $127 5 \pm 46$ for control titres by more than three standard deviations

The role of ascorbate in potentiating the production of

Table 2 Effect of ascorbate on interferon induction by poly(rI) poly(rC) in normal mouse embryo fibroblast cultures

Concentration of ascorbate (M)	Interferon titres*	
	Experiment 1	Experiment 2
None added	95	160
1 0×10⁻⁴	310	415
NIH reference	11,600	26,635

*Titres are expressed in DU_{50} U ml⁻¹

interferon is not clear Vitamin C may affect the interactions of poly(rI) poly(rC) at the cell surface or within the cell[4] Conceivably, ascorbate could function to increase the amount of interferon messenger RNA available for translation in cells stimulated with the polynucleotide by inhibition of a regulatory protein[5,6] and promotion of the stability of the interferon messenger RNA[7] There have been reports of stimulation of the antiviral activity of interferon by cyclic AMP[8] and, more recently, by cyclic AMP and certain of its synthetic derivatives[9] In this connection, preliminary experiments have suggested little difference in cyclic AMP levels between untreated and ascorbate-treated L cells as determined by radioimmunoassay[10] Cultures assayed after 4 h of exposure to ascorbate contained 60, 74 and 60 pmol mg⁻¹ nucleic acid, respectively, for cells untreated and treated with 10⁻⁵ and 10⁻⁴ M ascorbate In another experiment, cells assayed after 18 h of treatment presented cyclic AMP levels of 75, 77 and 72 pmol mg⁻¹ nucleic acid for the respective cultures The participation of ascorbate in enhancing interferon synthesis in mouse cells *in vitro*, as well as in the intact animal[1], remains to be elucidated

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Possible cocarcinogenic effects of coffee constituents

ALTHOUGH the carcinogenic properties of secondary N-nitrosamines and related compounds are well established[1,2], their causal relationship to human cancer remains an open question because very little is known about the degree of human exposure to them Carcinogenic N-nitrosamines have been detected in some nitrite-preserved foodstuffs, but only at relatively low levels[3,4], so the major uncertainty comes from their formation *in vivo* from ingested nitrite and amino compounds Formation of N-nitrosamines in this way in the digestive tract of laboratory animals has been widely demonstrated (for typical examples, see refs 5–7) and has led to the induction of tumours characteristic of N-nitrosamines[8,9] We have suggested[10] that the interaction between nitrite and phenolic materials should also be considered, because the latter are major dietary constituents which usually react much more rapidly than most amino compounds with nitrous acid Certain natural phenols may therefore inhibit N-nitrosamine formation both in foodstuffs and in the digestive tract We report here, however, that readily oxidised phenolic compounds act as catalysts rather than inhibitors for

N-nitrosamine formation from nitrite salts and secondary amines at gastric pH This finding has important implications on the possible cocarcinogenic properties of several foodstuffs and beverages, including coffee

Oxidation of *o*- and *p*-dihydroxphenols (and of *o*- and *p*-aminophenols) to the corresponding quinones is well known[11] and may be anticipated for HNO_2, itself an oxidising agent (standard oxidation potential at 298 K, $E° = -0 98$ V)[12] Our experiments confirm this expectation Thus, treatment of 4-methylcatechol (I), for example, with dilute (10⁻²–10⁻³ M) HNO_2 at pH 2–4 and 25 °C gives rapid formation of the corresponding *o*-quinone (II) with evolution of nitric oxide (equation) Since the oxidation of 4-methylcatechol seems to be complete in about 8 min, it must be one of the best known scavengers for HNO_2, but its presence, however, catalyses concurrent N-nitrosamine formation

In the absence of added phenols, the more basic secondary aliphatic amines such as dimethylamine and piperidine react sluggishly with aqueous HNO_2, even at pH 3–3 4, where the maximum rate is observed[13] for example, $t_{\frac{1}{2}}$ is about 10h for dimethylamine with [HNO_2]=0 1 M and 25 °C (ref 13) N-nitrosamine formation is even slower at high pH and our finding for piperidine (Table 1), giving $t_{\frac{1}{2}}$ about 160 h at pH 4, [HNO_2]=0 1 M and 25 °C, is typical In the presence of readily oxidised phenols, however, formation of the N-nitrosamine is very much faster Thus, typical results summarised in Table 1 show that addition of even 10⁻³ M 4-methylcatechol increases the rate of N-nitrosopiperidine formation by a factor of at least 1,000 In practice, significant amounts (about 10%) of N-nitrosopiperidine are found within the brief period (3 min) required to effect the first sampling, and its concentration reaches a maximum (Table 1) after about 10 min at 25 °C Increasing the temperature to 39 3 °C has little effect on the amount of N-nitrosamine formed but its formation rate, as would be expected, is increased Without the addition of 4-methylcatechol the amount of piperidine converted to the N-nitroso derivative after a reaction time of 10 min would be less than 0 1 % even with the highest [HNO_2] examined

4-Methylcatechol bears a close structural resemblance to the phenolic component of chlorogenic acid (III), a very substantial (about 13% by weight), soluble constituent of coffee[14] This compound (III) is therefore present in both 'instant' and ground coffee extracts to the extent of about 260 mg per cup[14] Our experiments (Table 1) show that (I) and (III) exert very similar catalytic effects on N-nitrosamine formation Thus addition of 10⁻² M (III) leads to a maximum 56% N-nitrosopiperidine formation in about 10 min at pH 4 and 25 °C compared with 42% for 10⁻² M (I) Further, we have found in preliminary experiments that a proprietary brand of 'instant' coffee, itself, when added to the buffered HNO_2–piperidine reaction mixture at a realistic consumptive concentration of 2 g per 100 ml, also leads to rapid and substantial formation of N-nitrosamines

Table 1   Rates* of N-nitrosopiperidine formation in aqueous phthalate buffers at 25 °C

pH	10^2 [NaNO$_2$] M	[Added phenol] M	$10^6 \, k_1* s^{-1}$	% N-nitrosopiperidine† after $t=10$ min‡
4 0	10	—	1 22	< 0.1
4 0	10	0 1 [I]	—	42
4 0	10	0 01 [I]	—	49
4 0	10	0 001 [I]	—	36
3.56	1.0	0 01 [I]	—	19
3 56	1 0	0.001 [I]	—	17 5
4 0	10	0 01 [III]	—	56 0
3 67	1 0	0 001 [I]	—	20§

* Rate$=k_1$ [Piperidine]
† Identified by ultraviolet, thin-layer chromatography and gas–liquid chromatography analyses
‡ Significant N-nitrosopiperidine formed almost immediately and reaches a maximum in about 10 min (see text) The amount given is the percentage of the initial [piperidine] (10^{-3} M)
§ At 39 3 °C, where the maximum percentage reaction shown is reached after 3 min

It is clear that both (I) and (III), under mildly acidic conditions, are very powerful catalysts for N-nitrosamine formation in aqueous media, and other readily oxidised phenols may be expected to act similarly The mechanism of their catalytic action is the subject of current investigation, but we believe that either nitric oxide or an aryl nitrite is the very reactive nitrosating species involved Significantly, oxidation of (I) and N-nitrosopiperidine formation both seem to be complete in about 8–10 min, and we have established that nitric oxide combines rapidly with piperidine under our experimental conditions.

The experimental conditions and reactant concentrations are not appreciably dissimilar from those expected for the human stomach following the ingestion of food containing 200 p p m $NaNO_2$ (about 3×10^{-3} M) and a single cup of coffee (about 7×10^{-3} M in chlorogenic acid) The clear implication is that coffee, and other foodstuffs containing readily oxidised phenolic materials, may significantly increase human exposure to carcinogenic N-nitrosamines by catalysing their formation in the digestive tract

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Tumour antigen specificity of a DNA-binding protein from cells infected with adenovirus 2

Two new polypeptides of molecular weights 75,000 and 45,000 are found in KB cells infected with human adenovirus type 2 (Ad 2), (ref 1) They represent the major labelled protein components of a nuclear membrane fraction ('DNA replication complex') that is capable of synthesising adenovirus DNA *in vitro*[1-3] It has been proposed that these polypeptides are early viral gene products, for they are synthesised soon after infection in the presence of 1-β-D-arabinosylcytosine (Ara C), an inhibitor of DNA synthesis[1] Their association with the DNA replication

complex suggested that they may have an affinity for DNA and could play a role in the replication of adenovirus DNA It was subsequently shown that these two polypeptides bind strongly to single-stranded DNA–cellulose columns and that large amounts of these 'DNA-binding proteins' can be isolated from the cytoplasm of the infected cells[2]. Two polypeptides of similar molecular weights (72,000 and 48,000) were independently isolated from cell extracts of monkey kidney cells, abortively infected with Ad 5, by their ability to bind to single-stranded DNA–cellulose[4,24] The use of temperature-sensitive mutants of Ad 5 indicated that these polypeptides are viral-coded[4,24]. Comparison of tryptic maps suggested that the 48,000 component was a degradation product of the 72,000 polypeptide (A J Levine, unpublished) A polypeptide of molecular weight about 65,000–75,000 has also been detected by polyacrylamide gel analysis in KB cells soon after infection by Ad 2 and Ad 5 (refs 5–7) and in HeLa cells infected by Ad 2 (ref 8)

Ad 2 and Ad 5 (together with Ad 1 and Ad 6) comprise the group C human adenoviruses[9] Members of this group transform rat cells *in vitro*[10], and DNA fragments of subgenomic size isolated from Ad 2 and Ad 5 have been reported to transform hamster, rat and human cells *in vitro*[11] Most transformed rat cell lines derived by infection with Ad 2 contain DNA sequences representing about 14% of the viral genome and contain tumour (T) antigens[12]. T antigens are virus-specific early proteins that are synthesised during productive infection as well as in transformed and tumour cells[8] They are generally detected by two immunological methods, complement fixation (CF) of cell extracts and immunofluorescence of fixed cells, using sera from hamsters bearing adenovirus-induced tumours. T antigens induced by adenoviruses within the same group cross-react serologically[9] We describe below the results of three types of immunological measurements—CF analysis, radioimmune precipitation, and radioimmune precipitation-inhibition—which show that the DNA-binding protein of molecular weight 75,000 (75k protein) in cells infected with Ad 2 has T-antigen specificity.

The 75k protein was purified from the cytoplasm of infected cells by chromatography on a single-stranded DNA–cellulose column as described[2] The bulk of the 75k protein is present in the fraction eluting at 0 6 M NaCl from the DNA–cellulose column (0 6 M eluate) By modifying our published procedure[2] (Fig 1), we obtained preparations of 75k protein free of the 45,000 molecular weight protein Analysis of polypeptides labelled with ³H-leucine in the 0 6 M eluate by polyacrylamide gel electrophoresis revealed a single major polypeptide peak of a molecular weight of 75,000 with only minor amounts of contaminating labelled polypeptides (Fig 1) Unlabelled 0 6 M eluates were also electrophoresed and shown to contain 75k protein as the predominant constituent after staining with Coomassie brilliant blue

Unlabelled 0 6 M eluates were assayed by a CF microassay using hamster sera against several different T antigens (anti-T sera) The anti-Ad 1 and Ad 2 T sera and ascitic fluid were prepared in hamsters by injecting extracts of transplanted hamster tumours induced by Ad 1–SV40 or Ad 2–SV40 hybrid viruses Anti-Ad 12 (group A) and anti-SV40 T sera were
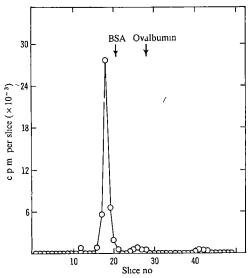
Fig 1 Gel electrophresis of DNA-binding proteins in the 0 6 M eluate from a single-stranded DNA–cellulose column KB cells were grown in suspension in Eagle's MEM containing 5% horse serum and were infected with Ad 2 at a multiplicity of 100 PFU per cell[2] At 2 h after infection, 25 µg ml⁻¹ of Ara C were added At 6 h, cells were centrifuged, washed with leucine-free MEM at 37 °C, and resuspended in leucine-free MEM with 5% horse serum Ara C (25 µg ml⁻¹) and ³H-leucine (2 µCi ml⁻¹, 41 Ci mmol⁻¹) were added and the cells were further incubated until 24 h after infection Cells were harvested by centrifugation, washed with phosphate-buffered saline, and resuspended in 0 01 M Tris, pH 7 4, 0 01 M NaCl, 0 0015 M MgCl₂ at a density of 10⁷ cells ml⁻¹ After standing in ice for 15 min, the swollen cells were disrupted by 15–20 strokes of a tight-fitting Dounce homogeniser and nuclei were removed by centrifugation at 2,000g for 10 min through a 5 ml cushion of 25% sucrose in 0 01 M Tris, pH 7 4, 0 01 M NaCl, 0 0015 m MgCl₂ The supernatant cytoplasmic fraction was treated with 20 µg ml⁻¹ of DNase I for 30 min at 22 °C in the presence of 0 05 M MgCl₂ and 0 001 M 2-mercaptoethanol The treated cytoplasm was dialysed overnight at 4 °C against at least 100 volumes of dialysis buffer containing 0 02 M Tris, pH 8 1, 10% glycerol, 0 05 M NaCl, 0 005 M EDTA and 0 001 M 2-mercaptoethanol A precipitate formed, which was removed by centrifugation before DNA–cellulose chromatography Single-stranded DNA–cellulose was prepared according to Litman[13] All DNA–cellulose column procedures were performed at room temperature Dried single-stranded DNA–cellulose (0 4 g) was suspended in dialysis buffer and used to pack a 1 8 cm³ column (0 8 × 3 0 cm) The column was washed with 10 ml of dialysis buffer and loaded with the cytoplasmic extract (approximately 24 mg protein) at a flow rate of 10 ml h⁻¹ The column was washed with 10 ml of dialysis buffer and eluted with 10 ml each of dialysis buffer containing 0 4 M NaCl and 0 6 M NaCl Bovine serum albumin (50 µg ml⁻¹) was added to the 0 6 M eluate to stabilise the DNA-binding proteins The 0 6 M eluate was concentrated by dialysis against 0 02 M Tris, pH 8 1, 0 05 M NaCl, 60% glycerol, 0 001 M EDTA and 0 001 M 2-mercaptoethanol overnight at 4 °C and was stored at −20 °C SDS gel electrophoresis was performed[14] with gels containing 6% polyacrylamide and 0 16% bis-acrylamide at 5 mA per gel for 30 min, then at 10 mA for an additional 6–8 h The gels were fractionated into 2 mm slices with a Gilson gel fractionator and counted in Aquasol (New England Nuclear) Marker bovine serum albumin and ovalbumin were electrophoresed in parallel gels and visualised by staining with Coomassie brilliant blue

obtained from hamsters bearing tumours induced by those viruses Typical results presented in Table 1 show positive fixation to a titre of 1 32 with anti-Ad 1 and Ad 2 T sera Anti-Ad 12 T serum, anti-SV40 T serum, and goat antiserum to purified Ad 2 virions were all negative Control preparations consisting of disrupted Ad 2 virions and a 0 6 M NaCl eluate from DNA cellulose chromatography of cytoplasm from mock-infected cells (Table 1, mock 0 6 M eluate) did not react with the anti-T sera These results indicate that the 75k protein is a non-virion, group C-specific T antigen found only in infected cells

CF titres of the 0 6 M eluate with anti-Ad 1 and Ad 2 T sera were relatively low and could perhaps reflect the assay of a minor contaminant rather than the 75k protein We therefore tested

labelled 0 6 M eluates in an indirect radioimmune precipitation assay modified from Horwitz and Scharff[18] Immunoglobulins isolated from the hamster anti-T serum and ascitic fluid and goat anti-virion serum were used rather than whole sera to facilitate comparison between different sera Since over 90% of the radioactivity in the 0 6 M eluate is present in the 75k protein, immunoprecipitation of more than 10% of the radioactivity would mean that the 75k protein and not a minor contaminant is specifically measured Two different labelled 0 6 M eluates were assayed, and the results of a typical experiment are presented in Fig 2 A maximum of 63–66% of the input radioactivity in the 0 6 M eluate was specifically immunoprecipitated with anti-Ad 1 and anti-Ad 2 immunoglobulin, whereas only 7–10% (same value as the normal immunoglobulin control) was precipitated by the immunoglobulins from the anti-SV40 T or goat anti-virion sera The lack of quantitative precipitation could be caused by labelled contaminants in the 0 6 M eluate, or by a major second protein comigrating with the 75k protein (two early viral mRNA molecules of nearly identical size that could code for the 75k protein were recently reported[20]), or by the presence
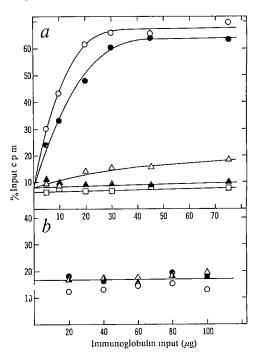
Fig 2 Radioimmune precipitation assay of 0 6 M eluates from Ad 2-infected and mock-infected cells for Ad 2 T antigen Immunoglobulins were isolated from hamster anti-T sera and ascitic fluid and from goat antiserum to Ad 2 virions by DEAE-cellulose chromatography and concentrated by precipitation with 50% saturated (NH₄)₂SO₄ (ref 19) The immunoglobulins were dialysed against PBS–EDTA (0 01 sodium phosphate, pH 7 2, 0 1 M NaCl and 0 005 M EDTA) The 0 6 M eluates were diluted in PBS–EDTA containing 1% desoxycholate and 1% Triton X-100 and clarified at 12 000g for 15 min before assay Constant amounts of labelled eluate were added to increasing amounts of the hamster or goat immunoglobulin in 300 µl PBS–EDTA containing 1% desoxycholate and 1% Triton X-100 Immunoglobins from a pool of normal hamster sera or a normal goat serum were added to each tube to provide a total of 125 µg of protein to maintain a uniform precipitate in all tubes Control tubes containing only normal hamster or goat immunoglobulin were used to determine the background level of nonspecific precipitation The mixtures were incubated at 37 °C for 30 min in siliconised 2 ml centrifuge tubes (Kimax) and 50 µl goat anti-hamster IgG or 60 µl pig anti-goat IgG sera were added (optimal proportions were predetermined in precipitation assays) The tubes were further incubated for 2 h at 37 °C and the precipitated complexes were sedimented at 900g for 10 min The precipitates were dispersed by mixing (Vortex), washed twice in PBS–EDTA containing 1% of desoxycholate and 1% Triton X-100, dissolved in 0 25 M acetic acid, and counted in Aquasol *a*, 0 6 M eluate from infected cells Input, 8,522 c p m, 10,000 c p m µg⁻¹ *b*, Mock 0 6 M eluate Input 4,834 c p m 550 c p m µg⁻¹ ○, Anti-Ad 1 T, ●, anti-Ad 2 T, △, anti-Ad 12 T, ▲, anti-SV40 T, □, goat anti-Ad 2 virions

Table 1 Complement fixation assay of the Ad 2 75k DNA-binding protein

| Source of antigen | Hamster anti-T sera against | | | | Goat antiserum against Ad 2 virions | |
	Ad 1	Ad 2*	Ad 12	SV40		
Ad 2 0 6 M eluate	1 32†	1 32	≤1 2‡	≤1 2	≤1 2	
Mock 0 6 M eluate	≤1 2	≤1 2	≤1 2	≤1 2	ND	
Disrupted Ad 2 virions	≤1 2	≤1 2	≤1 2	≤1 2	1 256	

The cytoplasm from cells infected with Ad 2 or mock-infected cells (treated in the same manner as infected cells but no virus was added) was fractionated on single-stranded DNA cellulose columns as described in Fig 1 Ad 2 virions purified as described[16] using CsCl for RbCl in the density gradient step were disrupted by the method of Prage et al[16] and sonicated before assay since the released cores tended to aggregate The 0 6 M eluates and the preparation of disrupted Ad 2 virions were assayed by the CF microassay[17] Pre-titred hamster and goat antisera were heated at 56 °C for 30 min and used at a dilution that gave five to ten units of antibody Assays were performed with 1 5 units of complement Similar results were obtained with a second preparation of 0 6 M eluate

* Ascitic fluid rather than serum was used
† Titre expressed as the highest dilution of antigen giving complete fixation
‡ The titre is 1 2 or lower, 1 2 is the anticomplementary titre of the antigens alone and represents the lower limit of the assay
ND, not done

of some denatured 75k protein that does not react with anti-T serum Some precipitation above background level was consistently observed with the anti-Ad 12 T immunoglobulin This could indicate that a low degree of cross-reactivity between T antigens of group A and group C is detected by the sensitive radioimmune precipitation assay A mock-infected 0 6 M eluate (labelled with a fivefold higher level of ³H-leucine since few host proteins are present in the 0 6 M eluate) was also assayed (Fig 2) This preparation did not react above the background level observed with normal immunoglobulin

The specificity of the radioimmune assay for T-antigen activity was demonstrated by radioimmune-inhibition experi-
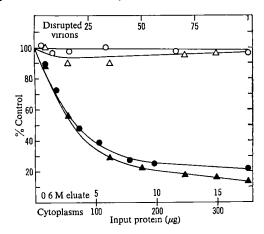
Fig. 3 Radioimmune-inhibition assay of Ad 2 T antigen activity In the inhibition reaction, aliquots of 125 μg of an immunoglobulin mixture from anti-Ad 2 T ascitic fluid (50 μg) and from a normal hamster serum pool (75 μg) were preincubated with increasing amounts of unlabelled 0 6 M eluate (infected cells), unlabelled cytoplasm from infected or mock-infected cells, or disrupted Ad 2 virions, prepared as described in Fig 1 Incubation was for 30 min at 37 °C in 400 μl of reaction mixture containing PBS-EDTA, 1% desoxycholate, and 1% Triton X-100 Control tubes consisting of the same mixture of immunoglobulins described above were preincubated with buffer alone ³H-eluate (13,049 c p m, 10,000 c p m μg⁻¹) was then added and the tubes were incubated for an additional 30 min Goat anti-hamster IgG serum (50 μl) was added and incubation was continued for an additional 2 h at 37 °C The precipitated complexes were then treated as described in Fig 2 and counted ○, Disrupted Ad 2 virions, ●, 0 6 M eluate (infected cells), △, cytoplasm from mock-infected cells, ▲, cytoplasm from infected cells The different protein inputs are given on the abscissae The degree of inhibition (% control) was calculated as follows after subtraction of background c p m (nonspecific precipitation with normal hamster immunoglobulin) from both inhibition and control reactions

$$\% \text{control} \quad \frac{\% \text{ input c p m precipitated in inhibition reaction}}{\% \text{ input c p m precipitated in control reaction}} \times 100$$

ments As shown in Fig 3, the precipitation of ³H-labelled protein from the 0 6 M eluate with a saturating level of anti-Ad 2 T immunoglobulin could be inhibited by 80% by preincubating the immunoglobulin with increasing amounts of unlabelled 0 6 M eluate or unlabelled cytoplasm from infected cells No inhibition was observed on preincubation with disrupted Ad 2 virions, or with the cytoplasm from mock-infected cells Note that a twentyfold higher input of infected cytoplasm was needed to obtain the same level of inhibition observed with the 0 6 M eluate This result indicates the feasibility of measuring 75k protein in unlabelled extracts of infected, transformed, or tumour cells by radioimmune-inhibition assay

No function has yet been assigned to the 75k protein A protein that binds to single-stranded DNA is required for the replication of bacteriophage T4 (ref 21) We have shown that the 75k DNA-binding protein fulfils the criteria for T antigen, as it reacts with antibody elicited to Ad 1 and Ad 2-transformed cells in hamsters It seems likely that there is more than one T antigen in Ad 2-transformed cells since several virus-specific RNA species have been detected[22] Carroll et al[23], have recently shown that a T antigen isolated from SV-40-transformed mouse cells binds to double, but not to single-stranded DNA It will be of interest to determine whether all Ad 2-transformed cell lines and tumours, as well as the rat line recently transformed by a 1 6×10⁶ molecular weight fragment of Ad 2 and Ad 5 DNA (ref 11), synthesise the 75k protein. This may indicate whether the 75k protein has an obligatory role in the induction and maintenance of the transformed state

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Paramagnetic species in cataractous human lenses

KURZEL *et al*[1] studied the low temperature phosphorescence emission from normal and cataractous human lenses at 77 K After comparing their lens data with those obtained from tryptophan in a frozen ethanol–water glass, they concluded that the phosphorescence of the lens is caused by the tryptophan residues of lens proteins This was based on their finding in the lens a phosphorescence λ(max), phosophorescence excitation and phosphorescence quantum yield consistent with tryptophan and differing significantly from those of phenylalanine and tyrosine Here we present the results of an electron spin resonance (ESR) study which characterises the paramagnetic triplet state of normal and cataractous human lenses and provides proof that it stems from the tryptophan moiety and not tyrosine or phenylalanine This supplements previous observations that tryptophan is the dominant species in cataractous human lenses, giving rise to phosphorescent emission In addition, we have discovered that the lens excited triplet (phosphorescent) tryptophan is associated with the production of a free radical, we believe that this free radical may be the missing link between ultraviolet irradiation and lens photodamage leading to cataract formation[3,4]

ESR measurements were made with a Varian E-4 spectrometer The ultraviolet light source consisted of a Bausch and Lomb Model SP-200 mercury lamp which was focused by quartz optics on to the irradiation slits in the ESR cavity The microwave power was set at 10 mW or 100 mW for all ESR measurements and the microwave frequency ranged between 9 089 and 9 093 GHz

Cataractous lenses were obtained intact from surgery and noncataractous *post mortem* lenses were obtained intact at autopsy The lenses were frozen immediately in liquid nitrogen and stored at 77 K Before ESR measurements, the lenses were thawed, and each lens nucleus was removed and inserted into a quartz sample tube (3 mm inner diameter), the samples were then refrozen to 77 K

Aromatic amino acid samples (0 1 mM) were prepared by dissolving them in solvents consisting of equal volumes of 0 1 M phosphate buffer (pH 7 0) and glycerol The solutions were placed in quartz sample tubes (3 mm inner diameter) and frozen to 77 K For ESR measurements, the samples were placed in a liquid nitrogen-filled finger Dewar which was subsequently inserted into the microwave cavity

The triplet state ESR absorption derivative obtained in this study corresponds to the $\Delta M = 2$ transition The position of the peak, H_{min}, on the low field side of the spectrum is related to the zero-field-splitting parameter which characterises the spin–spin interaction between the two electrons which comprise the triplet H_{min} and the linewidth of the ESR signal are both sensitive to the electronic structure of excited molecules and can be used to identify the triplet species
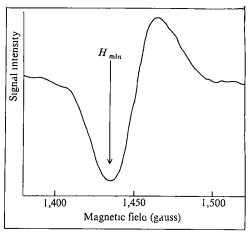
Fig. 1 Triplet state of ESR spectrum for the $\Delta M = 2$ transition in the human lens Temperature=77 K Microwave frequency= 9 092 GHz Microwave power=10 mW
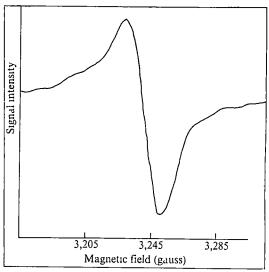
A typical triplet state ESR spectrum for the human lens is shown in Fig 1 For a microwave frequency of 9 091 GHz, H_{min} was 1,435±5 gauss and the peak-to-peak linewidth was 26±2 gauss No difference in either H_{min} or linewidth was observed between normal and cataractous lenses

For the frozen tryptophan, tyrosine and phenylalanine solutions at 77 K, we obtained H_{min} and linewidth values of 1,430±5 gauss, 1,240±5 gauss, 1,225±5 gauss and 25± 2 gauss, 30±2 gauss, 60±2 gauss, respectively We take the close agreement between the lens and tryptophan parameters as conclusive evidence that the lens protein phosphorescent state originates from the tryptophan residues, Shiga and Piette[5] reached a similar conclusion for ovalbumin and bovine serum albumin

The triplet state lifetime of both normal and cataractous lenses was then measured, a difference would be interpreted to reflect an alteration in the microenvironment of tryptophan between normal and cataractous lenses[6] The signal-to-noise ratio in the finger Dewar was not adequate to measure triplet state lifetimes, therefore, a Varian Model E257 variable temperature accessory was used At 133 K, the phosphorescent triplet state in both normal and cataractous lenses was found to have a lifetime of 4 0±0 1S

While making our ESR measurements, we observed that the intensity of the triplet state signal from both lenses and tryptophan solutions decreased after prolonged ultraviolet irradiation Along with the decrease in signal intensity, a gradual brunescent coloration developed in the samples which had previously

Fig 2 ESR spectrum of the ultraviolet induced free radical in the human lens Temperature=77 K Microwave frequency= 9 092 GHz Microwave power=100 mW The *g* factor=2 005

been uncoloured. We believe this coloration can be attributed to the presence of free radical products.

To test for the formation of free radicals, we irradiated a normal lens sample in the low temprature ESR finger dewar. We were able to follow the growth of the free radical species shown in Fig. 2. We have not been able to identify the radical species but assume that it results from the two photon ionisation of tryptophan. This has been demonstrated previously in frozen tryptophan solutions[7].

Human lenses are known to become more coloured with age and contain an increased quantity of insoluble protein, leading to loss of transparency. Current theories suggest that this coloration and decreased transparency may be related to photo-induced changes in aromatic amino acids[2,6,9]. Recently, photo-oxidation products of tryptophan have been identified in the human lens[10,11]. Our data suggest that a mechanism for photoinduced lens damage might be the production of free radicals. Roubal and Tappel[12] have shown that free radicals induce protein polymerisation and loss of solubility. Free radical reactions have also been implicated in ageing [13].There is reason to believe that ageing and cataractous lenses present the proper conditions for the stabilisation of free radicals. We have shown that ascorbic acid, which is a known free radical scavenger, is lost from the lens nucleus during cataract formation (unpublished data). The percentage of hydrophobic proteins also increases in the ageing lens[14], which may permit trapping of free radicals[15].

The possibility that ultraviolet light may be implicated in photodamage to the human lens is important, requiring further study. This is especially necessary in view of the widespread use of ultraviolet light today.

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Electron diffraction studies of biological membranes and lipids

IN diffraction studies of biological membranes, X-ray diffraction has been used more frequently than electron diffraction in spite of certain advantages inherent in the latter technique. Electron diffraction enables the study of a small area (about 1 μm^2) of a very thin specimen, and the collection of experimental data within a short time. Moreover, the morphology and degree of purity of the specimen can often be investigated. It seems possible to overcome the main problems arising from the application of the electron diffraction technique to biological materials, namely the difficulty of studying hydrated materials and the damage caused to the specimen by the electron beam, and a study of wet biological membranes has been reported[1]. In this investigation dry specimens have been studied. Radiation damage was largely overcome by using
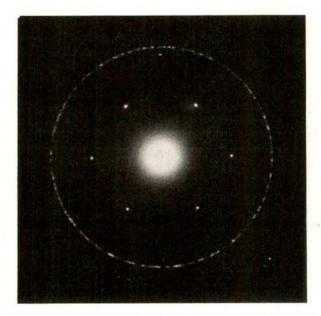
Fig. 1 Diffraction pattern of an unfixed and unstained A. laidlawii membrane. The two rings originate from the aluminium support film. The organisms were grown in a lipid-depleted basal medium supplemented with elaidic acid[2], and the membranes were isolated by osmotic lysis and washed[3]. After the last washing the membranes were resuspended in 10 mM ammonium acetate and deposited on copper grids covered with a 10 nm thick support film of aluminium. Membrane lipids were extracted with chloroform–methanol (2:1), freed from non-lipid contaminants[4] and fractionated into neutral, phospho- and glycolipids on a silicic acid column (BioSil HA). Lipid specimens for diffraction studies were prepared by spreading a lipid solution on a water surface and depositing the film obtained on grids, which were either bare or covered with an aluminium film. Stearic acid specimens were prepared in the same way.

minimum beam current, by maximally overfocusing the first condenser of the electron microscope, and by moderately overfocusing the second condenser. To minimise the temperature rise of the specimen, caused by inellastically scattered electrons, a cooled specimen holder was used. To facilitate heat transport, the specimen grids were covered with aluminium instead of carbon. A Philips 301 G electron microscope equipped with an anti-contamination device was used and the diffraction patterns were recorded at 100 kV on a highly sensitive X-ray film, Kodirex. All diffractograms were recorded at a specimen holder temperature of about −60 °C, which was regarded as a fair estimate of the temperature of the specimen itself.

Diffraction patterns of Acholeplasma laidlawii membranes (Fig. 1) showed the same pattern geometry as the stearic acid and the isolated lipids. The patterns were obtained only from small observation areas, about 1 μm^2. Nevertheless, superimposed, disoriented patterns could occasionally be seen. When larger areas were chosen, the observed patterns transformed into typical powder diagrams. The disoriented patterns might have derived from superimposed membranes or from a few domains within the same membrane possessing different orientations. The powder diagrams obviously originated from several membranes present in the observation area.

The diffraction patterns showed three spacings: 2.49, 3.67 and 4.15 Å, with an interplanar angle of 90° between the 2.49 and 3.67 reflections, and an angle of 68° between the 4.15 Å reflections. These data suggest an orthorhombic unit cell having an a axis of 7.37 Å and a b axis of 4.98 Å. Larger and slightly less reproducible spacings, about 2.6, 4.1 and 4.6 Å, were observed in preparations washed with buffers containing calcium or magnesium ions. It therefore

seems reasonable to assume that the presence of divalent cations caused the larger spacings in less exhaustively washed membranes. Interactions between these ions and the phospholipids would thus be the most feasible ones.

To investigate the influence of dehydration on the structure of the lipids in the *Acholeplasma* membranes, an X-ray powder diffractogram was taken, using a Guinier camera. The same spacings as in the diffractograms described above were obtained. This indicates that the water content of the membrane is of minor importance for the lipid structure below the thermal transition temperature.

The distances and angles characterising the diffractogram presented here are rather close to the parameters of the proposed hexagonal structure of biological membranes above the transition temperature[1,3,6]. The latter structure could be obtained by small changes in the 3.67 and 2.49 Å distances reported above. Thus our results show that diffraction studies of dry, single membranes in the static state prevailing below the thermal transition temperature are of importance for our understanding of the dynamic state characterising the membranes above this temperature. The finding that the 3.67 Å reflection is probably caused by lipids and not by proteins as proposed earlier[5] should also be mentioned in this connection.

We thank Dr S. Andersson for the use of the Philips EM 301 G microscope and for discussions, and Professor C. Weibull for reading the manuscript.

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Primordial origins of chirality

THE origin of optical asymmetry in relation to the origin of life has been extensively discussed[1-4]. One suggestion is that there is a connection between molecular asymmetry and the asymmetry of elementary particles produced by weak interactions. Various mechanisms have been proposed whereby the choice of one optical isomer could be influenced by longitudinally polarised (left handed) electrons from β decay[5,6], but early experiments did not give any significant results[7]. Later, both positive[8,9] and negative[10-12] results have been obtained in a variety of experiments (differential decomposition of enantiomers bombarded by β⁻-rays, spin-polarised positron and muon annihilation in enantiomers).

In a new approach to this problem we carried out the crystallisation of DL-NaNH₄ tartrate in the presence and absence of chiral β^- particles from ^{32}P. Below 27 °C, DL-NaNH₄ tartrate crystallises in racemic conglomerates[13], that is, D and L crystallisation centres form independently and then grow. Any difference between the number of D and L centres will have a more marked effect on the optical activity of the conglomerates as crystallisation proceeds.

Pure DL-tartaric acid, free of mesotartaric acid, was con verted to the sodium ammonium salt. Its optical activity was checked by measuring α_{280} with a JASCO ORD/UV-5 spectropolarimeter, and θ_{225} with a JASCO 40c dichrograph. The sensitivity of the latter is ± 0.1 m°, and a 1×10^{-6} M excess of one isomer could have been detected. In the starting racemic material 2×10^{-5} M predominance of D (+) isomer was found.
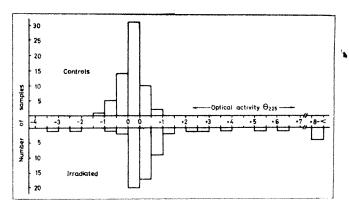
Fig. 1 Distribution of levels of optical activity in NaNH₄-tartrate samples as crystallised in the absence (upper part of diagram) and in the presence (lower part) of chiral β^- particles (63 crystallisations in both cases). Positive CD signal at 225 nm (θ_{225}) corresponds to a negative rotation at the sodium D line, which is characteristic of the unnatural L (−) isomer.

For crystallisation, 8 ml 45% solutions of the tartrate were used. In six series (in all, 63 independent crystallisations) K₃^{32}PO₄ was added, the level of radioactivity being 20 μCi ml⁻¹; in the six control series, inactive K₂H^{31}PO₄ was added. The pH in all series was adjusted to 9.5 with NH₄OH. After equilibration at room temperature crystallisation was carried out over P₂O₅ in a desiccator at 1–4 °C. Precautions were taken in cleaning the glassware, excluding dust, and so on. When the crystallisation was about half complete (8–12 d), the crystals were filtered and weighted, and their optical activity measured in 2×10^{-2} M aqueous solution. As a check, the optical activity of the supernatant was also measured.

Figure 1 shows that in the presence of chiral electrons (^{32}P) the unnatural L (−) salt crystallised preferentially whereas an approximately symmetrical distribution is obtained in the control series, in the series with ^{32}P there is a markedly asymmetric distribution. It is clear, however, that β^- particles shifted the crystallisation towards L (−) isomer. The shift is significant at the 0.1% level (both χ^2 and Student's t test). The magnitude of the shift is indicated by the medians: 0.0 for control series, and 0.2 for irradiated samples. As would be expected, the small predominance of the D (+) isomer in starting material very slightly shifted the crystallisation of the control samples towards D (+) isomer.

The exact mechanism of the effect is not known. We think it unlikely that β rays would influence crystal growth, and suggest that the influence was to enhance the number of crystallisation centres of the L isomer. In this connection it should be mentioned that hydrated electrons have quite a long lifetime, particularly in alkaline solutions[14].

We thank Dr Cs. Fajszi, Dr. Soós and L. Kovács for their help.

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reviews

To review one volume of Needham's great monument-in-the-making is like trying to make an architectural assessment of a single pillar of a temple. But one is encouraged in the attempt because a pillar is not a bad analogy. The superstructure of modern science is sustained on many singular supports; Greek atomism, mediaeval astronomy, Renaissance navigation, and so on. One of the pillars, alchemy, changes its appearance as one changes one's viewpoint.

There has been a lot of change in the historical assessment of alchemy. For a time it was viewed as the main forerunner of modern scientific chemistry; then as mere superstition and charlatanism; then as a lesser light to technological chemistry. Now we are beginning to re-assess it yet again as something respectable in its own right, a body of practice and belief that should be viewed not in terms of modern science and modern society, but as something of its own time. It is one of the outstanding merits of Needham's work that at the same time as he makes us look for the first time at a culture we did not pretend to know, he also makes us look afresh at the western science we thought we did know. This is true in general of his whole enterprise, but particularly clear in this volume.

It is at once chastening and stimulating to see Chinese alchemy studied with an eye to chemical detail which has not been applied so closely by any writer about western alchemy in the present generation of historians of western science.

Alchemy was concerned with many things, but the big thing was the distinctive character of gold. Gold was astonishing stuff to the ancient world. Familiarity with it never bred contempt, because one characteristic marked it off from all else; it was incorruptible. It survived all trials and could be recovered pure and undiminished from alloys and disguises by the process of cupellation. True: it was beautiful, but other things were beautiful. The imitation of its beauty was one thing: aurifiction, the production of the appearance of gold. But could gold be made: was true aurifaction a possibility? And if one form of matter could be incorruptible, could not Man be made incorruptible? So far, so simple. These are the familiar themes of western alchemy. The value

Science ancient and modern

Frank Greenaway

Science and Civilisation in China. Vol. 5: *Chemistry and Chemical Technology.* Part 2: *Spagyrical Discovery and Invention: Magisteries of Gold and Immortality.* By Joseph Needham with Lu Gwei-Djen. Pp. xxxii + 510. (Cambridge University Press: London, October 1974.) £12.00; $35.00.

of Needham's work lies in his expounding Chinese alchemy as a thing distinct in itself, having its own moral values, social relations and methods of enquiry. If some resemblances, some identities, are discovered which show Chinese and western alchemy to have a common basis and to have arrived at some common conclusions, this is for a good reason: "While we can easily see that artistic styles and expressions, religious ceremonies and doctrines, or different kinds of music, have tended to be incommensurable; for mathematics, science and technology the case is altered—man has always lived in an environment essentially constant in its properties, and his knowledge of it, if true, must therefor tend towards a constant structure".

Is the limited career of Chinese alchemy to be considered in Spenglerian terms, an aspect of a quasi-organic cultural growth and decay, or in Kuhnian terms, an aspect of the adoption of a compelling fashion of thought in a line of succession without progress? Needham respectfully rejects the Spenglerian pessimism but will not accept a naive Kuhnism. In fact, he will not accept entire any of the currently packaged philosophies of history, but insists that he is engaged in an enquiry which will lead to a new appreciation of the origins, development and internal and external relations of Chinese science when the work is done. His study of Chinese science leads him to remind us how provisional is our modern scientific 'knowledge'.

"It is neither independent of the accidents of Western European history, nor is it a final court of appeal for the eschatological judgment of the value of past scientific discoveries

either in West or East. It is a reliable measuring stick so long as we never forget its transitory nature."

One of the most difficult exercises for the historian of science is to relate the techniques of a historical period to modern scientific and technical knowledge. Needham's treatment of the metallurgical practices which the alchemists used for precious and base metals is exemplary. These pages can be recommended to any reader, whether he cares about Chinese alchemy or not, who wants a brief, logical (and so far as I can gauge, pretty complete) survey of all early metallurgical developments. There are some surprises, such as the widespread use of cupronickel, the as yet unexplained occurrence of some specimens of aluminium, and the extent of the trade in zinc.

When Needham has to deal with the derivative human theme of alchemy, the elixir of life, the drug of deathlessness, he does so by relating it most skilfully to the technical, metallurgical theme. He shows how the idea of physical immortality emerged almost imperceptibly out of the idea of longevity. There is, however, no one persistent view to be shown. There were many religions in China, influential successively or simultaneously, and each had its own philosophy of the nature of human life and its prolongation. In each, alchemical ideas could play a part. The descriptions of religious observance, incense burning in particular, are very well done, the accounts of unfamiliar liturgical and ritual customs never being obscured by the inevitably complex detail.

The excellence of the scholarly apparatus of these successive volumes is well known and needs no further praise. Nor does Needham's unfailing courtesy and generosity to his collaborators, whether they contributed to a whole chapter or a single line.

The value of this volume, only one of the four projected for the whole of Chinese chemistry, is likely to be as much in its stimulus to the reappraisal of western alchemy as in its original *raison-d'être*. A successful and convincing history of scientific chemistry as a whole has yet to be written. It would be strange, yet somehow satisfactory, if the stimulus to a new approach to a critique of modern chemical science should come from this splendid study of things "far away and long ago".

Biology of the gene

Gene Expression. Vol. 1 : *Bacterial Genomes.* Pp. xvii + 642. June 1974. £4.75. Vol. 2 : *Eucaryotic Chromosomes.* Pp. xiv + 467. September 1974. Benjamin Lewin. £8.00, cloth; £3.95, paper. (Wiley, London and New York.)

GENE EXPRESSION, originally intended as a revision of Benjamin Lewin's earlier successful treatise, *The Molecular Basis of Gene Expression,* reflects the growth of knowledge in the field and has expanded to become two major volumes.

Volume 1, *Bacterial Genomes,* reviews the extensive progress in the molecular genetics of prokaryotes over the past five years. The coverage is not limited strictly to gene expression, but includes the closely allied topics of recombination, DNA replication and cell division. The biochemically biased exposition, intended for advanced students and specialists, demands familiarity with both microbial genetics and nucleic acid biochemistry.

Lewin presents his subject through a critical appraisal of the key experiments, occasionally including carefully selected data. The clarity of the presentation is enhanced by the well designed diagrams. Original papers are abundantly cited; curiously though, references to the decisive experiments seem too often absent.

Volume 2, *Eucaryotic chromosomes,* contrasts strikingly with Volume 1 in the lack of precise information deriving, quite naturally, from the relative complexity of the subject. Lewin argues that sufficient advances have been made in our knowledge of eucaryotic gene expression to justify this attempt at proposing "a preliminary conceptual framework".

The first part of Volume 2 is devoted to the ultrastructure of eucaryotic chromosomes and its variation during the life cycle. The very lengthy discourse palls at times, but is alleviated by many excellent electronmicrographs. The analysis of DNA structure from the kinetics of renaturation and hybridisation is well explained, but the need for a sharper tool with which to approach sequence organisation in eucaryotic DNA is apparent between the lines.

The second part of the book is more directly concerned with the expression of eucaryotic genes. The processing of primary transcripts and models for the control of eucaryotic gene expression are prominent. The volume ends with an interesting discussion of the interaction between nucleus and cytoplasm, as studied by nuclear transplantation and somatic cell hybridisation.

Despite Lewin's apologies for his selectivity, in both volumes he could reasonably have been more selective and thereby more concise. Certain experiments described in great detail are superseded by subsequent reports. Even elegant experiments which have been conclusively refuted are best forgotten. In each volume, after such an array of experimental observations and conclusions, I would have welcomed an attempt to present a wider view, putting recent developments into perspective and discussing their implications for the future. I felt it a pity, too, that the concentration on mammalian cells in Volume 2 meant the omission of those eukaryotic systems most amenable to genetic analysis and, therefore, most likely to yield important information on gene structure and expression.

These minor criticisms should not be taken to detract seriously from the obvious merit of these volumes, the depth, scope and modernity of which will ensure their value to everyone interested in the biology of the gene.

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Watching volcanoes

Physical Volcanology. (*Developments in Solid Earth Geophysics,* vol. 6.) Edited by L. Civetta, P. Gasparini, G. Luongo and A. Rapolla. Pp. xvi + 333. (Elsvier Scientific: Amsterdam, Oxford and New York, 1974.) Dfl.90; $34.75.

THIS book represents the combined efforts of four editors and seventeen contributors to mark the occasion of the retirement in 1971 of Professor Giuseppe Imbò from the directorship of the Vesuvian Observatory.

Like all commemorative books it is very conservative; thus we see again the classic investigations of volcanic seismicity from Japan and of crustal deformation from Hawaii.

That wouldn't matter if the UNESCO book on the same subject had not been published in 1971, but this new book repeats (verbatim in some instances) some 30% of that material.

And the title seems to me to be wrong. The book deals mainly with the application of instrumental techniques in all but two of the fourteen chapters, and though that makes for an excellent reference book, it rather avoids consideration of the underlying physics.

I think that a more interesting volume could have been written under the title of 'Physical Volcanology' if some attention had been given to the physical processes of volcanic phenomena. The physics of lava flows, cone formation, and magma degassing are hardly mentioned, and the more avant garde subjects like the influences of earth tides and local tectonics on eruptions are not seen.

The book, however, contains some very useful reference material for anyone actively engaged in volcanic research. Most of the chapters describe in detail the application and limitations of one or other of the geophysical and chemical techniques that have been successfully used on volcanoes, in each case by one of the contributors, all of whom are acknowledged experts. It is to be hoped, though, that they are not led by the title of Chapter 9 into confusing ashes for gases; and I hope too that their efforts may help avert the great ions (p. 317) resulting from large lava flows. **James L. Brander**

Centenarian from a village near Sukhumi on the Black Sea coast. From *Biological Anthropology* (Readings from *Scientific American*). With Introductions by Solomon H. Katz. Pp. 494 + 340 illustrations. (Freeman: San Francisco, 1975.) $14.95, cloth; $6.95, paper.

More than most will remember

Collected Papers of Sir Harold Jeffreys on Geophysics and Other Sciences. Vol. 2: *Observational Seismology;* Pp. xxi + 697; £20.10. Vol. 3: *Gravity;* Pp. xviii + 661; £19.60. (Gordon and Breach, London, Paris and New York, 1975.)

HAROLD JEFFREYS is truly one of the giants of 20th century science, although he ploughed a fairly lonely furrow between the wars at a time when bright young men were expected to go into atomic and nuclear physics. But now geophysics is the most fashionable of subjects and many of its practitioners rely, consciously or unconsciously, on the foundations that Jeffreys built. His writings both on geophysics and beyond (even to an appraisal of the psychoanalytic significance of a label on a beer bottle) have been both prolific and a delight to read. The seven textbooks are easily accessible, but the rest is scattered around more than 40 different journals. So is there maybe a case for the publication of his collected papers?

For all that Jeffreys has published widely, four fifths of the papers in these volumes (of the six that will ultimately appear) come from the *Monthly Notices of the Royal Astronomical Society* and its *Geophysical Supplement* (later the *Geophysical Journal*). (There were times when Jeffreys practically kep.' the *Geophysical Supplement* going single-handedly!) Thus, if you have access to a complete run of these two journals you are paying a lot for the other one-fifth of his published papers. And the editing is relatively slight.

One of the fascinations of science is the way things age rapidly—particularly the interpretation of observations; it is, then, of interest to learn what, in retrospect, scientists would stand by, and what they think is no longer tenable. For instance, I should have liked to know how Jeffreys thought the J-B tables of 1940 stood now that there are newer tables by Herrin (at least, I do know, but I wish he'd put it in print). But where Jeffreys does add a modern footnote—and this is infrequently—it is brief and not very helpful. For example: "Some stations, mainly southern ones, received much higher reliabilities when the ellipticity of the Earth was allowed for", at the end of a 20 page paper.

One footnote, though, is pure Harold Jeffreys and touches on the question of the WKBJ approximation. In a 1969 note on a section in a 1915 paper, Jeffreys writes "This was my first discovery of what is best described as the Green–Liouville approximation. I

rediscovered it in 1923, having twice forgotten Green's equivalent treatment of tidal waves in a canal of slowly varying sections". He has forgotten more than most of us will remember.

Alas, the price of the complete set is such that the only geophysicists with that sort of money to spare are not those who will think of acquiring these volumes. And librarians can with justification say that all the papers are accessible somewhere—except perhaps the beer-bottle one.

David Davies

Marihuana

Marijuana: Effects on Human Behaviour. Edited by Loren L. Miller. Pp. xiv + 405. (Academic: New York and London, December 1974.) $29.50; £14.95.

The Use of Marihuana: A Psychological and Physiological Inquiry. Edited by Jack H. Mendelson, Michael Rossi and Roger E. Meyer. Pp. x + 202. (Plenum: New York and London, 1974.) £17.00; $44.00.

OVER the last few years, research on cannabis has burgeoned astonishingly. This has not come about by the slow process of natural evolution, but very obviously because grant money has flowed in that direction, particularly in the United States and Canada. For anyone with a really well developed eye for a bandwagon, the ultimate would now be to obtain grant money for a study of the social and political determinants of this granting. The story would provide a fascinating case-study.

When, for basically political reasons, a great deal of research money is suddenly pushed in a particular direction the prognosis is always uncertain—the result may be a lot of mediocre research or, alternatively, rather good things may happen. With research into the effects of cannabis the outcome has been of the latter and more fortunate kind. In several different scientific areas cannabis research is beginning to have about it a modest feeling of excitement.

The range of this research and something of the promise, is well conveyed in the volume on cannabis which is edited by Dr Loren Miller. Edited volumes on cannabis are now appearing thick and fast, but so far this is undoubtedly the most interesting and authoritative array of papers on this topic to have been put together in one book. The 14 chapters each either report the contributor's original research with adequate delineation of context, or review a particular sector. The laboratory dissection of the psychological aspects of cannabis is the theme

of several different chapters, and one feature of recent work is that experiment on the drug's interference with complex mental functions is providing a useful tool for basic study of those types of function. Chapters on the influence of cannabis on memory and on attention, nicely illustrate this development. Other contributions at the laboratory end of the spectrum deal with neurophysiological research and possible interference with neurotransmitter mechanisms; again these are not just 'drug studies' but use of a particular substance as an investigating tool. We have moved on from the days when study of CNS function was largely based on physical ablation of its parts, but the analogy is close.

This book also deals with some more social–medical questions. A chapter on the drug and psychiatric illness, gives a particularly sensible appraisal of issues which have not always attracted the coolest discussion. Progression to other drugs, cannabis and violence, and cannibis and car driving, provide the matter for further chapters. Too easy an acceptance of the inevitability of that approach to research which is always one removed from the social reality (drinking experiments, as it were, in the simulated laboratory bar rather than at the Pig and Whistle), receives a healthy jolt from an account of driving research in which experimental subjects drove round town in dual control cars after smoking, with careful real-life observation being backed by physiological measures made at the same time.

In *The Use of Marihuana* Professor Mendelson and his colleagues give an account of a project which yields a mass of data on a number of separate aspects of the drug's effect on the human subject. Even leaving aside the specialism of the content, this is a book which deserves notice as a superbly competent piece of scientific investigation. Some years ago Mendelson and his collaborators made an important contribution to alcoholism studies by observation of, and multiple measures on, alcoholics drinking in an experimental ward, which provided an operant-conditioning paradigm: the same type of design has now been applied to the influence of cannabis on man. In tolerant individuals, marihuana intoxication sustained over a period of 3 weeks and with dose on occasion going up to 200 mg THC a day, produced remarkably little disturbance. As the investigators themselves say, their work leads to no closure of the cannabis debate but only points to the necessity for "more sophisticated questions". Any assertion that cannabis is a drug which cannot produce tolerance, is after this study certainly no longer credible.   **Griffith Edwards**

Prosimians and evolution

Prosimian Biology. (Proceedings of a Meeting of the Research Seminar in Archaeology and Related Subjects.) Edited by R. D. Martin, G. A. Doyle and A. C. Walker. Pp. xxi+983. (Duckworth: London, November 1974.) £30.00.

"THIS collection of papers" the late W. C. Osman Hill wrote in the foreword, "can be seen to cover an extremely broad spectrum". He was far from wrong. Fifty four chapters by authors from many countries and several disciplines describe the behaviour, ecology, physiology, anatomy, neurophysiology, cytology, biochemistry and evolution of prosimians.

The book represents more than an addition to current knowledge of another group of little known mammals. As Doyle and Martin point out in their introductory chapter, the morphology of contemporary prosimians is broadly similar to that of the array of Eocene primates from which the Anthropoidea developed. Living species may thus provide an approximate blueprint of the behaviour and ecology of the common ancestors of prosimians and anthropoids. Moreover, the many examples of convergence between prosimian and anthropoid species occupying similar niches strengthen arguments relating behavioural and morphological differences to variation in ecology. For both reasons, the study of prosimians may help us to understand the evolution of the higher primates.

The book's most important contribution lies in the first section. This reports the results of field studies covering 15 different species. Until recently, the behaviour and ecology of prosimians was poorly documented, partly because most species are nocturnal and almost all live in habitats where observation is difficult. This group of papers represents a major advance in the field and bears witness as much of the authors' endurance as to their perspicacity. The extensive sections on anatomy, biochemistry and evolution provide many insights into the functional significance of particular structures, ranging from teeth to toenails, and into the phylogenetic status of a wide variety of species. A general weakness, considering the stated justification for studying prosimians, is the authors' reluctance to discuss the relevance of their findings to the evolution of the higher primates and man.

The book is well produced and carefully edited. It contains fewer makeweight chapters than most published collections of conference papers. And

Lesser bushbabies (*Galago senegalensis moholi*)

it is likely to represent a milestone in its field for many years. Nevertheless, it should make contributors, editors and publishers think carefully about the wisdom of producing similar volumes. At £30.00 it is beyond the reach of private buyers. All except the most affluent libraries are likely to look askance at highly specialised sympo-

sium proceedings which cost as much as a year's subscription to the average journal. If the size and cost of symposia proceedings continue to escalate at this rate, it is inevitable that they will only be available in the largest libraries. That will benefit neither readers nor authors.

T. H. Clutton-Brock

Most theories according to others

Transport Phenomena in Aqueous Solutions. By Tibor Erdey-Gruz. Pp. 512. (Adam Hilger: London; Akademiai Kiado: Budapest, December 1974.) £12.00.

THE transport properties covered in this book are the traditional ones of shear viscosity, concentration diffusion and electrical conductivity. There is only passing mention of bulk viscosity, thermal diffusion, dielectric relaxation, and so on. The three properties covered form the subjects of chapters 2, 3 and 4. The first chapter discusses the molecular structure of liquid water and the fifth, which should perhaps more logically have been the second, covers the equilibrium properties of aqueous solutions.

The author has an encyclopaedic knowledge of what has been said and done, but shows little discrimination. Every experiment and, what is worse, every theory is referred to, but he rarely tries to judge between them. Thus, sections 1.3.3.2 to 1.3.3.9 describe briefly eight different classes of theories of the structure of water and are followed, as if in despair, by 1.3.3.10 which is called simply "Further Theories". His classification is impeccable but he does not tell the reader which of these mutually inconsistent theories he

believes to be correct; nor does he marshall well the evidence on which the reader could judge for himself. The same is true of the treatment in later chapters on theories of transport. Moreover, even his references become perfunctory when he touches on modern theories which require for their understanding a knowledge of non-equilibrium statistical mechanics.

Perhaps he sees it as the duty of the author of a monograph simply to summarise the views of others (the commonest phrase in the book is "according to X"), and not to inflict his own views on his readers, but it makes for a dull book. The only point at which it comes alive is in the section (p. 351–390) on the effect of non-electrolytes on electrolytic conductivity. There, the author has himself contributed experimentally to the subject and his discussion is much more cogent than elsewhere.

The book can be recommended, however, to those who want access to the voluminous experimental literature, and to the mechanically more simple theories of the last 40 years. The references and indexes are, on the whole, accurate, comprehensive and organised in a way which makes it easy to recover the information in the literature.

J. S. Rowlinson

announcements

Awards

Her Majesty the Queen has approved the grant of a **Royal Charter** to the Institute of Measurement and Control.

The Royal Meteorological Society has announced the following awards **Symons Memorial Gold Medal** to **B. J. Mason, FRS** (development of meteorology and understanding of the physics of clouds), **Fitzroy Prize** to **R. W. Gloyne** (application of meteorology and climatology to agriculture, horticulture and forecasting), **L. F. Richardson Prize** to **J. J. Barnett** (stratosphere on Nimbus IV)

The Royal Society has announced the election of the following Fellows **R. J. H Beverton, CBE; G. M. Binnie, E. G. Bowen, CBE; S. H U. Bowie; G. M Brown; A. D. Buckingham; P. C. Caldwell; J. Charnley, CBE, J W. Christian; B. A. Cross; K. Dalziel, P. De Mayo; J. M. Dodd; A. Edrélyi; D. A Haydon; G. N. Hounsfield, A M. Lane; A. R. Lang; A. L. McLaren; R. Mason; C. Milstein; P. A. P. Moran; R. C. Rainey; E. C. Slater; R. A Slatyer, D. C. Smith; B P. Stoicheff, G. P. L. Walker; R Weck, CBE, F. R. Whatley, R. Wilson, E C. Zeeman.**

Appointments

Peter Palmer, a Director of Drake and Cubitt Holdings, has been appointed the first visiting professor in the department of mechanical engineering of the City University

James Briden has been appointed professor of geophysics in the department of earth sciences at Leeds University

International meetings

May 6–7, Isolation and clinical significance of trace components of plasma, Washington, DC (Symposium Planning Committee, American National Red Cross, Blood Research Laboratory, 9312 Old Georgetown Road, Bethesda, Maryland 20014)

May 13–14, Pulsed methods in photochemistry, London (Dr M A West, The Royal Institution, 21 Albemarle Street, London W1X 4BS, UK)

June 9–August 29, Frontiers of science —Gordon Research Conferences on a wide variety of physical and biological topics, running throughout the summer, New Hampshire and California Applications to Dr A M Cruikshank, Director, Gordon Research Conferences, University of Rhode Island, Pastore Chemical Laboratory, Kingston, Rhode Island 02881, Programme Summary from Women's Colby College, New Hampshire, New London, New Hampshire 03257

June 16–18, Mass spectrometry in biochemistry and medicine, Alghero, Sardinia (Alberto Frigiero, c/o Instituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157 Milan, Italy)

June 16–20, American Geophysical Union, Washington, DC (Shelton Alexander, 204 Mineral Sciences Building, Pennsylvania State University, Pennsylvania 16802)

June 17, Polymer science : achievements and prospects, Pittsburgh (Hershel Markovitz, Department of Chemistry, Carnegie–Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213) The symposium is in honour of Paul J Flory, the Nobel Laureate in chemistry, 1974

June 22–28, Immunochemistry of the cell membrane, Ravello, Italy (Dr P Comoglio, Department of Anatomy, Turin University, Corso M D'Azeglio 52, 10126 Torino, Italy, or Dr S Zappacosta, Fondazione Pascale Cancer Institute, Via M Semmola, 80131 Napoli, Italy)

June 23–July 4, Physics of quantum electronics, Santa Fe, New Mexico (Professor S F Jacobs, Optical Sciences Center, University of Arizona, Tucson, Arizona 85721)

June 25–27, Mercury, Pasadena, California (James A Dunne, Executive Secretary, First International Colloquium on Mercury, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove, Pasadena, California 91103)

June 30–July 1, Applications of enzymes, Exeter (Miss M V Auguste, The Chemical Society, Burlington House, London W1V 0BN, UK)

June 30–July 4, Marine natural products, Santa Barbara, California (Paul J Scheuer, University of Hawaii at Manoa, Department of Chemistry, 2545 The Mall, Honolulu, Hawaii 96822)

Reports and publications

Great Britain

Memoirs of the Royal Astronomical Society, Vol 78, Part 2 Equivalent Width and Rotational Velocities of Southern Early-Type Stars By L A Balona Pp 51–72 (Oxford and London Blackwell Scientific Publications, 1975 Published for the Royal Astronomical Society) [281

The Stable Isotope Project—Progress Report 1970–1973 (Publications Series C, No 13, October 1974) Pp iv + 16 (London Natural Environment Research Council, 1974) [291

Bulletin of the British Museum (Natural History) Geology, Vol 25, No 4 Cretaceous Faunas from Zululand and Natal, South Africa, Introduction, Stratigraphy By W J Kennedy and H C Klinger, Pp 273–315 + 1 plate (London British Museum (Natural History), 1975) £3 75 [291

Philosophical Transactions of the Royal Society of London A Mathematical and Physical Sciences Vol 277, No 1272 Coupling Coefficients and Tensor Operators for Chains of Groups By P H Butler Pp 545–585 UK £1 50 Overseas £1 60 Vol 277, No 1273 On the Use of the Breit-Pauli Approximation in the Study of Relativistic Effects in Electron-Atom Scattering By M Jones Pp 587–622 UK £1 30, Overseas £1 50 (London The Royal Society, 1975) [301

Proceedings of the Royal Irish Academy Vol 74, Section A, Nos 18–36 Spectral Theory Symposium Pp 133–310 £3 45 Vol 74, Section B No 28 Some Pennireteppra (Bryozoa) from the Visean on County Fermanagh with a Revision of the Generic Name By Foluso Olaloye Pp 471–506 + plates 15–22 £1 53 No 29 Enzyme Production by Bacillus polymyxa in an Extract of Peat I Preliminary Studies and the Effect of Added Carbon on Amylase and Protease Production By W M Fogarty and P J Griffin Pp 507–522 31p Nos 30 and 31 Enzyme Production by Bacillus polymyxa in an Extract of Peat II The Effects on Nitrogen and other Factors on Amylase and Protease Production III Amylease and Protease Production in Batch Cultures By P J Griffin and W M Fogarty Pp 523–548 46p (Dublin Royal Irish Academy, 1974) [311

Department of Industry Laboratory of the Government Chemist Report of the Government Chemist, 1973 Pp iv + 175 + 8 plates (London HMSO, 1974) £1 80 net [311

Medical Research Council The Toxicity of Plutonium Pp iii + 38 (London HMSO, 1975) 45p net [311

Government Statistics a Brief Guide to Sources Pp 18 (London Central Statistical Office, Great George Street, SW1, 1975) [32

Mineral Resources Consultative Committee Mineral Dossier No 9 Tin Compiled by D Slater Pp vi+41 65p net Mineral Dossier No 10 Talc Compiled by D E Highley Pp 25 55p net (London HMSO, 1974) [32

University of Cambridge Annual Report of the Institute of Astronomy, 1973/1974 Pp 20 (Cambridge The Observatories, The University, 1975) [42

Philosophical Transactions of the Royal Society of London B Biological Sciences Vol 270, No 901 A Physiological Analysis of Walking in the American Lobster (Homarus americanus) By D L Macmillan Pp 1–59 (London The Royal Society, 1975) UK £2 50, Overseas £2 60 [62

Bird Life in the Royal Parks, 1973 (A Report by the Committee on Bird Sanctuaries in the Royal Parks) Pp 13 (London Royal Parks Division, Department of the Environment, 29 Great Peter Street, SW1, 1975) gratis [62

Materials for Cryogenic Service Engineering Properties of Austenitic Stainless Steels Pp 48 (London International Nickel, Ltd, 1974) [62

Estuaries Research Report of the NERC Working Party on Estuaries Research (Publications, Series B, No 9) Pp 62 (London Natural Environment Research Council, 27/33 Charing Cross Road, WC2, 1975) [62

Royal Observatory Bulletins No 178 Provisional Positions of the Sun and Planets 1957–1971 By M E Buontempo, J V Carey and Patricia Eldridge Pp 191–300 (Herstmonceux Royal Greenwich Observatory, 1973) £2 25 net [72

Bailey's Technical Dictionaries Catalogue 1974 Pp 80 (Folkestone Bailey Bros and Swinfen, Ltd, 1975) 70p [72

Ministry of Agriculture, Fisheries and Food Agricultural Chemicals Approval Scheme Insecticides Fungicides/Herbicides 1975 List of Approved Products and their Uses for Farmers and Growers Pp 182 (Harpenden, Herts Agricultural Chemicals Approval Organization, Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, 1974) [102

The Institution of Gas Engineers Discussions of the 111th Annual General Meeting, Bristol, 1974 (D931–940) Pp 151 (London The Institution of Gas Engineers, 1975) £1 [102

Other countries

Royal Ontario Museum Life Sciences Occasional Papers, No 26 Thermal Relations of the Neotropical Frog *Hyla labialis* (Anura Hylidae) By D Valdivieso and J R Tamsitt Pp 12 60c Life Sciences Contributions No 99 A New Genus of Freshwater Triclad from Tasmania, with Reviews of the Related Genera *Cura* and *Neppia* (Turbellaria, Tricladida) By Ian R Ball Pp 48 $2 No 100 A Revision of the Latipinnate Ichthyosaurs of the Lower Jurassic of England (Reptilia Ochthyosauria) By C McGowan Pp 30 $1 50 No 102 Fauna and Correlation of the Ravenscrag Formation (Paleocene) of Southwestern Saskatchewan By Loris S Russell Pp 52 $2 A Selected Bibliography on Mercury in the Environment, with Subject Listing By Susan Robinson and W B Scott Pp 54 $1 25 (Toronto Royal Ontario Museum, 1974) [271]

Canada Department of Energy, Mines and Resources Geological Survey of Canada Paper 74–9 Trace Element Variations in Porphyry Copper Deposits, Babine Lake Area, B C By J L Jambor Pp v + 30 $2 50 Paper 74–60 Computer Use in Projects of the Geological Survey of Canada By Terry Gordon and W W Hutchison Pp v + 108 $3 Miscellaneous Report No 21 Guide to the Geology of Prince Albert National Park—a Story of Hills, Lakes and Beaches By A H Lang Pp vii + 40 $2 Miscellaneous Report No 22 Guide to the Geology of Elk Island National Park—The Origin of its Hills and other Scenery By A H Lang Pp vii + 31 $1 50 (Ottawa Information Canada, 1974) [271]

CERN — European Organization for Nuclear Research CERN 74–25 Effet sur la Racine Principale de Vicia Faba de l'Exposition aux Rayons Gamma du 60Co, aux Protons de 600 MeV et aux Neutrons de 14 et de 400 MeV Par J M Laurent Pp vi + 122 (Geneva CERN, 1974) [281]

Smithsonian Contributions to Zoology, No 172 The So-called Cheirodontin Fishes of Central America with Descriptions of Two New Species (Pisces Characidae) By William L Fink and Stanley H Weitzman Pp iii + 46 (Washington, DC Smithsonian Institution Press, 1974 For sale by US Government Printing Office) $1 05 [281]

Australia Commonwealth Scientific and Industrial Research Organization Division of Food Research—Report of Research, 1973/1974 Pp 93 (Sydney CSIRO, Division of Food Research, 1974) [291]

Anuario del Observatprio Astronomica de Madrid para 1975 Pp 414 (Madrid Observatorio Astronomico, 1974) 100 pesetas [301]

Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Division of Tropical Agronomy, 1973/1974 Pp 168 (Brisbane, Qd CSIRO, 1974) [311]

Centre National pour l'Exploitation des Oceans Publications du CNEXO, Série Résultats des Campagnes a la Mer, No 08—Resultats de la Campagne Mediprod III (13 Juin-2 Juillet, 1972) Pp 21 (Paris CNEXO, 1974) [311]

Canada Department of Energy, Mines and Resources Geological Survey of Canada Bulletin 234 Evolution of a Middle and Upper Devonian Sequence from a Clastic Coastal Plain-Deltaic Complex into Overlying Carbonate Reef Complexes and Banks, Sturgeon-Mitsue Area, Alberta By L F Jansa and N R Fischbuch Pp 105 $5 Memoir 371 Geology of Tetagouche Lakes, Bathurst, and Nepisiguit Falls Map-Areas, New Brunswick, with emphasis on the Tetagouche Group By Ralph Skinner Pp 133 (42 plates) $8 (Ottawa Information Canada, 1974) [32]

Organization for Economic Co-operation and Development OECD Informatics Studies No 7 Information Technology in Local Government a Survey of the Development of Urban and Regional Informations Systems in Five European Countries Pp 168 (Paris OECD, London HMSO, 1974) 20 francs, £2, $5 [32]

International Institute of Cellular and Molecular Pathology—Descriptive Booklet Pp 199 (Bruxelles International Institute of Cellular and Molecular Pathology, Avenue Hippocrate, 1975) [32]

Saving Our Small World (Monday Conference on Population, Ecology and Resources) Pp 131 (Sydney The Australian Broadcasting Commission, 1974) $2 50 [32]

Scripps Institution of Oceanography Annual Report year ending June 30, 1973 Pp 64 (San Diego Scripps Institution of Oceanography, University of California, 1974) [32]

On Unification, Part 3 By Sakhawat Hossain Pp 47–72 Supplement Pp 14 (Dacca, 5 Sakhawat Hossain, 267 Elephant Road, 1972) [32]

Marine Science Teaching at the University Level—Report of the Unesco Workshop on University Curricula (Unesco Technical Papers in Marine Science, No 19) Pp 27 (Paris Unesco, 1974) [32]

New Trends in Integrated Science Teaching Education of Teachers Edited by P E Richmond Pp x+227 (Paris The Unesco Press, 1974) [52]

Australia Commonwealth Scientific and Industria Research Organization Land Research Series No 33 Explanatory Notes to the Geomorphological Map of Papua New Guinea By E Loffler Pp 19+ map (4 sheets) (Melbourne CSIRO, 1974) [62]

Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Division of Animal Health for 1973 Pp 119 (East Melbourne CSIRO, 1974) [62]

Carnegie Institution of Washington Report of the President, 1973/1974 Pp 32 (Washington, DC Carnegie Institution of Washington, 1975) [72]

United States Department of the Interior Geological Survey Professional Paper 805 Lower and Lower Middle Devonian Rugose Corals of the Central Great Basin By C W Merriam Pp v+83+ plates 1–25 (Washington, DC Government Printing Office, 1974) $2 85 [721]

Science Council of Canada Background Study No 32 Technology Transfer in Construction By A D Boyd and A H Wilson Pp 163 (Ottawa Information Canada, 1974) $3 50 [102]

Canada Department of Energy, Mines and Resources Geological Survey of Canada Bulletin No 218 Keweenawan Volcanic Rocks of Michipicoten Island, Lake Superior, Ontario, and Eruptive Centre of Protherozoic Age By R N Annells Pp 141 (22 plates) (Ottawa Information Canada, 1974) $5 [102]

Forschungsberichte des Landes Nordrhein-Westfalen, Nr 2449 Beziehungen Zwischen der Entropiefreien, der Chemischen und der Rationalen Thermodynamik der Vorgange Von Wolfgang Kern, Gunter Weiner und Prof Josef Meixner Pp 39 (Dusseldorf Westdeutscher Verlag, 1974) DM 5 20 [112]

Annual Report of The Experiment Station of the South African Sugar Association, 1973/1974 Pp 58 (Mount Edgecombe, Natal Experimental Station of the South African Sugar Association, 1974) [122]

Person to Person

Amentiferae collections. Anyone willing to collect regularly material for a floral development study of Myricaceae, Juglandaceae and Fagales is asked to contact as soon as possible Dr Alastair D Macdonald, Biology Department, Lakehead University, Thunder Bay, Ontario, Canada P7B 5E1

Newcastle–Bristol Furnished/unfurnished house or ground-floor flat required central Bristol or Frenchay area from June 1–December 31 For rent or exchange with four-bedroom house in Newcastle for same or shorter period (Professor J W Thompson, Department of Pharmacological Sciences, Medical School, The University, Newcastle upon Tyne NE1 7RU, UK)

There will be no charge for this service Send items (not more than 60 words) to Robert Vickers at the London office The section will include exchanges of accommodation, personal announcements and scientific queries We reserve the right to decline material submitted No commercial transactions

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European Organization for Nuclear Research—CERN CERN 75–1 Calculation of the Neutron-Induced Background in the Gargamelle Neutral Current Search By W F Fry and D Haidt Pp v+19 (Geneva CERN, 1975) [132]

Indian Forest Bulletin (New Series), No 266 Use of Insecticides for Pest Management in Forests By V P Sharma Pp 63 Rs 3 50, 41p, $1 26 No 268 Effect of Treating Conditions on the Anti-Shrink Effect and Bulking Attained in Wood by Urea and Sorbital Soak Treatments By S N Sharma, N R Das, R C Bhatnagar and R C Lohani Pp 19 Rs 2 10, 24p, 76 US cents No 269 Cleavage Properties of Some Indian Timbers By A C Sekhar and A S Gulati Pp 9 Rs 2, 30p, 94 US cents No 270 Chemical Seasoning of heavy and Refractory Axlewood (*Anogeissus latifolia*) Using Polyethylene Glycol–600 By S N Sharma, M L Mehra and S P Badoni, Pp 26 Rs 1 45, 18p, 54 US cents Indian Forest Leaflet No 195 Effects of Grain Alignment of Wooden Disc-Dowels Made from Radial (Quarter-Sawn) and Tangential (Plain Sawn) Planks on the Strength of Structural Joints By N J Masani and K S Pruthi Rs 2 20, 26p, 80 US cents (Delhi Manager of Publications, 1974) [172]

Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture Bruxelles Comptes Rendus de Recherches, No 38 Travaux du Centre de Recherches sur les Maladies Parasitaires des Animaux Domestiques Pp 154 (Bruxelles IRSIA 1974) [172]

Environment Canada Fisheries and Marine Service Technical Report No 499 An Automatic Monitor for Detecting and Recording Passage of Transmitter-fitted Fish By D G Pincock, D McG Luke, D W Church and A B Stasko Pp vi+59 (St Andrews, New Brunswick Research and Development Directorate Biological Station, 1974) [172]

United States Department of Agriculture Index-Catalogue of Medical and Veterinary Zoology Supplement 18, Part 6 Parasite-Subject Catalogue—Subject Headings and Treatment By Martha L Walker, Jane D Rayburn, Judith M Humphrey and Dorothy B Segal Pp iii+339 $2 95 Supplement 19, Part 6 Parasite-Subject Catalogue—Subject Headings and Treatment By Martha L Walker, Jane D Rayburn, Judith Humphrey Shaw, Margie D Kirby and Shirley J Edwards Pp iii+497 $4 60 Supplement 18, Part 7 Parasite-Subject Catalogue—Hosts By Shirley J Edwards, Margie D Kirby, Lila R Crawley, Jane D Rayburn, Judith Humphrey Shaw and Martha L Walker Pp xv+672 $6 05 Supplement 19, Part 1 Authors—A to Z By Dorothy B Segal, Judith M Humphrey, Shirley J Edwards, Margie D Kirby, Martha L Walker, Jane D Rayburn, Lila R Crawley and J M Podani Pp xviii+783 $7 35 (Washington, DC Government Printing Office, 1973 and 1974)

Bibliographien des Deutschen Wetterdienstes Nr 28 Agrarmeteorologische Bibliographie 1973 Bearbeitet von Maximilian Schneider Pp xxvii+246 (Offenbach a m Selbstverlag des Deutschen Wetterdienstes, 1974) [182]

Institute of Radiation Breeding, Ibaraki-ken, Japan Gamma Field Symposia No 11 Chemical Mutagenesis in Micro-organisms and Plants (Report of Symposium held on July 25 to 26, 1972) Pp 96 No 12 Induced Mutation and Chimera in Woody Plants (Reports of Symposium held on July 26 to 27, 1973) Pp 120 (Ohmiya-machi, Ibaraki-ken Institute of Radiation Breeding, NIAS MAF, 1972 and 1973) [182]

Deutsches Hydrographosches Institut, Hamburg Jahresbericht 1972/1973 Pp 158 (Hamburg Deutsches Hydrographisches Institut, 1974) [182]

The Open Universities in South Africa and Academic Freedom, 1957/1974 A Review by the Academic Freedom Committees of the University of Cape Town and the University of the Witwatersrand, Johannesburg Pp x+49 (Cape Town and Johannesburg Juta and Co, Ltd, 1974) [192]

United States Department of Agriculture Index-Catalogue of Medical and Veterinary Zoology Special Publication No 3 Ticks and Tickborne Diseases I Genera and Species of Ticks, Part 1 Genera A-G Pp 429 Part 2 Genera H-N Pp 593 Part 3 Genera O-X Pp 329 By Mildred A Doss, Marion M Farr, Katharine F Roach and George Anastos (Washington, DC Government Printing Office, 1974) [192]

Smithsonian Contributions to Astrophysics, No 17 Period, Colour, and Luminosity for Cepheid Variables By Cecilia H Payne-Gaposchkin Pp 10 50 cents Smithsonian Contributions to Zoology, No 187 Communication in Red Fox Dyads—a Computer Simulation Study By Gerald Gene Montgomery Pp iii+30 95 cents (Washington, DC Smithsonian Institution Press, 1974 For sale by US Government Printing Office) [202]

United States Department of the Interior Geological Survey Professional Paper 866 Tectonic Features of the Precambrian Belt Basin and their Influence on Post-Belt Structures By Jack E Harrison, Allan B Griggs and John D Wells Pp iii+15 (Washington, DC Government Printing Office, 1974) 65 cents [202]

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Please, SIPRI, stay on the fence

As commentators never tire of pointing out, the presence of the word 'peace' in the title of any organisation immediately renders the intentions of that body suspect, such has been the misuse of one of the most beautiful as well as one of the most significant words of the English language But the same commentators generally make that point in order to draw attention to the one glowing exception—the Stockholm International Peace Research Institute (SIPRI)—an organisation which in nine years has built up for itself a major reputation as a purveyor of accurate unvarnished information on armament and disarmament provided by an international team whose commitment has been to objectivity in assessment rather than to politically motivated public statements

Scientists have had particular cause to be grateful to SIPRI Much of the present debate on arms control and disarmament centres on technological feasibility, prospects held by military research and development and methods of verification In these fields, in which no national military organisation exactly invites the participation of outsiders, SIPRI has fought bravely to keep abreast and to provide hard fact The SIPRI Yearbook has become necessary reading for all who profess an interest in defence, of whatever political hue

Since SIPRI s inception, *Nature* has been both an admirer of the institute and a regular user of its data (This partly stems from the concerns of both the present and the immediate-past editor in nuclear matters The present editor (declaring an interest) also worked for a time at SIPRI) We shall, no doubt, continue to depend heavily on SIPRI for many things, but it is necessary to note a gradual shift in the institute's policy which could jeopardise its future effectiveness, at least if it conceives of its future function as similar to the one it has discharged with distinction in the past nine years
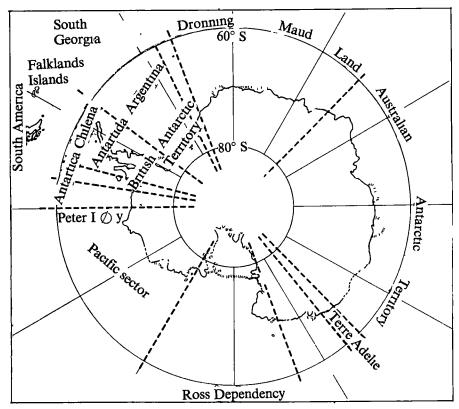
When SIPRI was founded by the Swedish government and guaranteed its independence, there were those who saw it simply as a private research department of Mrs Alva Myrdal, the Swedish delegate to the Eighteen-nation Disarmament Conference Certainly Mrs Myrdal has used SIPRI's work more than most, but any fears that the institute would become subservient to a particular philosophy were soon allayed As well as eschewing external alignments, SIPRI has had to watch its internal scene rather carefully Of necessity staff who are prepared to uproot their lives elsewhere in order to spend two or three years in Stockholm are going to be of more than average commitment The institute has never made any secret of this—indeed in the first yearbook the then director, Robert Neild, acknowledged that his staff were of one mind that the arms race was dangerous and that efforts to slow it down had been incommensurate with the danger SIPRI's skill has been in converting this conviction into objective analysis

In the past few years there have been signs of growing impatience—not with objective analysis but with political systems which stoke up the arms race and treat arms control with such little respect Until recently this impatience has hardly manifested itself in very extreme forms, the pages of SIPRI reports don't exactly drip with blood There has just been the odd paragraph or two in a preface or a summary indicating despair, particularly with the pursuits of partial arms control measures to the exclusion of more general disarmament Small beer, maybe, compared with the sort of editorialising that everyone else does—and why shouldn't they use their own results more positively? And yet the surprise one feels at encountering even such inoffensive (though controversial) comment is akin to the shock that would be created if the Registrar-General were to preface his annual statistics by a comment that he was getting tired of recording increases in population, people must cut down on copulation

The most recent publications from Stockholm suggest that the trend towards a more committed viewpoint continues Two booklets by Frank Barnaby, the present director, (*Preventing nuclear-weapon proliferation* and *Nuclear disarmament or nuclear war?*) are fairly obviously aimed at a new and much wider audience The emotional temperature has gone up a fair bit, to the extent of a few half-page pictures, not all strictly relevant (such as a napalm victim) and some ringing phrases about nuclear strategy being "inhumane, immoral even genocidal" and so on

Well, who are we to snipe about emotional writing and irrelevant pictures? The point is simply this If SIPRI is to become more polemical it will undoubtedly gain in popular acclaim and will always find a ready audience and willing supporters But it will forfeit its status as an organisation that can work within the system and command respect and help from insiders There are far too few bodies of this sort in arms control for this to be seen as anything but a matter of great concern

It is quite likely that much of this change of attitude at SIPRI springs from a feeling of impotence in the face of so much military expenditure This is to adopt too pessimistic view of the benefits of reliable factual reporting, for which the dividends are always less tangible but ultimately more profound And SIPRI has had real successes, particularly in its reporting on the world arms trade and its long term study in chemical and biological warfare These very successes have sprung from the institute's ability to seek opinions and deal directly and honestly with many individuals deeply involved in questions of defence and armament Come down off the fence on the other side from them and they will neither give advice nor read what is written □

Antarctica: the end of an era?

THE problems of mineral exploration in Antarctica will almost certainly be discussed when signatories of the Antarctic Treaty meet in Oslo in June Largely concerned with the potentially vast reserves of oil and other fossil fuels, the discussions will ensure that old issues of territorial sovereignty will once again be raised But fears that each of 12 signatory states intend to grant themselves exclusive rights of exploitation may be unnecessarily pessimistic, writes Alan Piper

There is still a considerable amount of doubt about the full extent and location of fossil fuel resources in Antarctica, though consideration of the pre-drift, Gondwana continental arrangements suggests that the Ross Sea and Weddel Sea areas may prove productive Even vague possibilities, however, lend an added significance to the debate about possible mineral exploitation in Antarctica and it is unlikely that the tacitly acknowledged, underlying political rationale behind the maintainence of expensive scientific bases in Antarctica can remain suppressed for very much longer

At the time that the Antarctic

The Antarctic Treaty, applying to the entire area of the globe south of 60°S, was signed in Washington DC on December 1, 1959 by Argentina, Australia, Belgium, Chile, France, Japan, New Zealand, Norway, South Africa, the Soviet Union, and the United States After ratification by all 12 signatory states the treaty entered into force on June 23, 1961, since when it has been acceded to by Czechoslovakia, Denmark, the Netherlands, Poland, and Rumania

Recognising that "it is in the interests of all mankind that Antarctica shall continue for ever to be used exclusively for peaceful purposes and shall not become the scene or object of international discord" the treaty
● suspends previously existing disputes over territorial sovereignty in Antarctica

● prohibits the establishment of military bases, the explosion of nuclear devices and the disposal of radioactive waste within the treaty area
● invests contracting parties with the right to inspect the full scope of another nation's scientific and logistic operations to encourage open scientific cooperation in Antarctica
● calls for regular consultative meetings between the signatory states for the purpose of "exchanging information, consulting together on matters of common interest pertaining to the Antarctic, and formulating and considering measures in furtherance of the principles and objectives of the treaty"

In 1991, after the treaty has been in force for 30 years the contracting parties will be required to review its operation

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Treaty (see box) was signed five of the contracting nations — Australia, the UK, France, New Zealand, and Norway—had previously asserted recog nised claims to about 80% of the Antarctic landmass and adjoining con tinental shelf in sectors radiating out wards from the pole Of the remainin signatories, Argentina and Chile ha advanced conflicting claims to most c the British sector, while the goverr ments of the Soviet Union and th United States had refused at any tim to recognise any territorial claims o the continent and have asserted non themselves, Japan had renounced he former territorial claims under th terms of the 1951 peace treaty

The treaty is justifiably held in hig regard as a considerable politic achievement It not only froze th legal *status quo* regarding claims t territorial sovereignty but, significantl: was the first multinational treaty c substance to involve the Soviet Unioi The demilitarisation of the Antarct continent and the provision for sciei tific cooperation has provided a coi tinuing zone of contact between th super powers

The treaty has worked well in assurance of exclusively peaceful oper tions And though each of the si natories can inspect "the full scope another nation's scientific and logisti operations", a right regularly exercis by several countries, no evidence h yet been produced to suggest that tl spirit of the treaty has been contr vened by any party

Regular consultative meetings of si natories are called for by the treat and at the last meeting, held in We lington in 1972, the representativ recommended to their governmen that "the subject 'Antarctic Resourc Effects of Mineral Exploration' 1 carefully studied and included on tl Agenda of the Eighth Consultati Meeting" The issue will certain figure prominently in the Os discussions

To date, there have been no sig that the UK has finalised a policy the issue of economic exploitation, b some decisions will have to be reach soon The obvious need is for a regin within which mineral exploitatio would be available to any state oper ing under licence, whether or not party to the present treaty Th approach to the problem, howeve would presuppose that the over-ridii issue of territorial jurisdiction had be settled As long as uncertainties ov sovereignty continue, it is doubtf whether signatories would agree to 1 cognise the legal rights of any outsid to operate within a claimed sector

It is to be hoped, at the least, that system of licensing under the contr of the present signatories will

adopted. Some of the signatories will arrive in Oslo with no definitive policy in mind. That is almost certainly true in the case of the United States. The likelihood of unilateral action, which has always remained a possibility with that country, cannot as yet be ruled out but the Americans will probably adopt a wait-and-see attitude: "On paper, the United States has a policy; in reality, it does not", says a recent article in *Science*. Whatever policies or

non-policies the signatories take to Oslo, however, there is little hope that they will be published in advance. When preparatory discussions, called especially to consider the problem of mineral exploration, ended in Oslo recently no formal statement was issued.

It would be unrealistic to expect that a settlement will be reached during the Oslo talks. (Discussions on the conservation of the Antarctic seals continued for 10 years before the final proposals

were adopted in 1972. Recommendations on measures for the conservation of Antarctic flora and fauna, first proposed in Brussels in 1964, have still not met with final approval.) But most of the parties to the treaty are unwilling to roll an already snowballing problem further into the future, and many will feel that further prevarication on their part will lead outside countries to question the capability of the signatories to handle the matter satisfactorily.   ☐

Limits to oncology

Dr Michael Stoker, Director of the Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, thinks that a levy on money for cancer research should be used to ensure that sufficient 'strategic research', as opposed to 'tactical research', is carried out. (From a lecture given at the dedication of the Seeley G. Mudd Building, M.I.T., on March 6.)

EVEN in these hard times, the support available for cancer research is relatively large by comparison with other biomedical fields, especially in the USA. But, in spite of clear enough aims in terms of alleviation of suffering caused by cancer, the course to be followed is uncharted and the weighting to be put on the various alternatives is largely guesswork. Indeed, the modest progress in prevention and cure of cancer has so far been based almost entirely on empirical judgements and serendipity, followed by well organised development, but it has given no guide to formulation of the principles to be followed for any major and general advance. Close monitoring and direction by the sponsors is, therefore, scarcely possible or at any rate useful. This results in a dilemma concerning the limits of cancer research supported by earmarked funds, particularly for those tackling the more

fundamental aspects of cancer biology.

I shall first consider, as background, some pressure which tend to broaden the limits of cancer research. At an extreme would be the socioeconomic viewpoint, which would consider cancer as a minor problem compared with poverty, population control, pollution and so on. This radical view could consider it justified, and not at all dishonest, to divert cancer funds to the greater good, or to choose research topics, which, though related to oncology, also contribute to the solution of greater social ills. But this almost certainly neglects the wishes of the donors. These may be based on emotion and irrational fears about the implacability of cancer, but it is surely the worst and most dangerous sort of scientific conceit to decide that you know better than the customer what he really wants, at any rate in terms of final objectives.

Then there are those who accept the objective of cancer alleviation, but for whom the modest progress so far, and the lack of a rational approach, means that a better understanding of biological systems as a whole is a necessary prerequisite for any real advance in cancer research. Cancer funds at this stage, therefore, should go predominantly to general biology. This viewpoint is no less strong through its reinforcement by the more personal motives and ambitions of scientists. Most of us would like to do something about cancer, but we are also strongly attracted to the great unsolved problems of wider generality, where advances will bring us the acclaim of our peers.

It is against this background, and the opposing forces of accountability, cost benefit and so on, that the question of relevance, and also responsibility to the sponsor or donor, must be considered. If you gave your money to cancer research, how would you like it spent? At one extreme almost any science, or at any rate biological science, would be relevant, whereas, at the other, cancer research funds should be restricted to programmes with at least conceptual links to practical applications in a finite time.

In some fields of research this dilemma can be acute, for example,

those relating to the molecular and supramolecular organisation of eukaryotic cells. Two main classes of research can be identified in relationship to oncology, and it is helpful to distinguish them.

The first would include any research programme which might have a bearing on cancer, but which is just as likely to lead to benefits in other fields of medicine, agriculture and so on. In other words there is no *a priori* reason to suppose that the resulting advances in knowledge will benefit cancer over and above any other human good. Clearly this research includes the general understanding of living systems and is very long term in its objectives. It is still applied research in that the goal is human health rather than satisfaction of curiosity, but it is multipurpose, with oncology included.

The second class covers research likely to be more relevant to cancer than anything else. Though other benefits may come, the reason for the research is the special likelihood of increasing the understanding and finally the alleviation of cancer, and not of other human ills. It may still be very much concerned with fundamental aspects of living processes, but those thought to be especially important in cancer.

I shall refer to the former approach as strategic research and the latter as tactical research. They can be distinguished by applying the following questions. Is the research programme under consideration more likely to benefit cancer than other ills? If so, it is tactical. Or does the research include cancer among other possible benefits? It would then be strategic. This requires judgement of outcome and not intent.

In the light of these criteria, I shall mention briefly three research fields which are at present believed to be of high priority and which compete for cancer research funds.

The first is the cell surface, which is at present a subject of such intensive study, much of it with cancer funds. But is the elucidation of the mysteries of the cell surface more likely to benefit cancer than for example the vascular diseases or the primary immunological disorders? Of course there are a num-

ber of changes to the cell surface which are considered to be characteristic of cancer cells, or at least of transformed cells; on closer examination, however, many, even of these, have been found to be manifestations of rapid growth and are almost certainly secondary rather than causal phenomena. This has led to an increasing concentration on the role of the surface in normal cell functions, in relation to both growth and differentiation. It does not diminish the possibility that a change in the cell surface is a primary event in a cancer cell, nor should one forget the particular importance of the tumour-specific surface antigens, but the main thrust of cell membrane research seems now to be much more concerned with normal cell biology; although it is of the utmost importance and one of the most fruitful of all present fields I would nevertheless class a good deal of it as strategic research rather than tactical in its relationship to cancer.

Second, tumour virology is clearly concerned more directly with cancer than anything else and is therefore tactical research. This is not only because such viruses are carcinogens but because of their quite exceptional additional values as models of eukaryotic genetic material containing cancer genes. Nonetheless, it may turn out that the ubiquitous endogenous viruses are as relevant to normal development as to cancer. RNA-directed DNA synthesis, which is now used for all sorts of problems, could carry wide implications over and above its special role in certain tumour viruses, and it does not necessarily follow that all reverse transcription is oncology.

Finally, consider differentiation, a diverse and popular field of research which is particularly difficult to assess in relation to cancer. Differentiation involves changes in gene expression and since cancer also is now widely believed to be a manifestation of gene regulation, strong claims are made for cancer funds for support of differentiation, morphogenesis and so on in a variety of prokaryotic as well as eukaryotic systems. Indeed, there is now an additional justification for cancer funds, with the use of the teratoma as a model system. But it does not follow that even a full understanding of gene expression, for example, in embryogenesis, will necessarily lead to a solution of that other change in cell regulation which leads to cancer. In fact, many would hazard a guess that there is a fundamental difference: namely, that cancer involves a change in the genome and differentiation does not.

Most studies on development and differentiation must surely be classed as strategic research, with possible relevance to cancer as well as to many other practical problems.

Other examples, such as chromosome structure, protein synthesis and so on, would show that there is really a continuous gradient of research in terms of relevance to cancer. Nonetheless, I would maintain that a position on the gradient can usefully be identified at which cancer changes from one of many potential beneficiaries to the primary potential beneficiary. There will naturally be sharp differences of opinion as to where this point lies in relation to specific research programmes and many will disagree with the examples given. But that does not diminish the general value of distinguishing strategic and tactical research when considering allocation of cancer funds.

It would obviously be simple and administratively tidy to restrict cancer funds to tactical research alone, and to leave the support of strategic reseach to other sources. It would also be an evasion of responsibility and, in my view, a serious mistake. The vast accumulation of knowledge about cancer and cancer cells is matched only by our abysmal lack of understanding, which simply reflects our inability to solve the complexities of eukaryotic cells. So cancer research funds must surely contribute in some way to strategic research dealing with these fundamental issues. But the method of funding should not encourage deceit and need not necessarily be the same as for the more straightforward tactical aspects of oncology.

There have been many studies of the relationship between fundamental research and useful application, mostly in the physical sciences and engineering, and it is generally agreed that a simple causal chain between a curiosity-oriented discovery in a university laboratory and the emergence of a useful product or other advance is, to say the least, extremely rare. The relationship is complex, and there is much to be said for Harry G. Johnson's concept of basic research contributing to a general pool of knowledge in which the applied scientist or developer fishes and takes samples as he needs them (*Minerva*, **XI**, 17; January 1972). The result is a lack of temporal correlation between the acquisition of the elements of knowledge and their subsequent appearance in useful application.

My plea therefore is that some mechanism should be found so that cancer funds can be used for the maintenance and expansion of this pool of general knowledge. What I am proposing, in effect, is a sort of levy or tax on cancer money to help pay for its dependence on strategic biology. It was Rothschild who included, in a controversial report a few years ago, an enlightened and more widely applicable proposal along these lines: namely, that most government laboratories, whatever their objectives, be allowed about 10% of their overall budget for allocation to more basic research, not strictly accountable in terms of relevance. The levy from cancer funds might perhaps be higher but we should not forget the sucesses of the past decade or so, in Hodgkin's disease for example, chorioncarcinoma and some leukaemias, and in at least the avoidance of lung cancer. These advances have not depended, so far as I can see, on any deep understanding of the molecular basis of livng processes, and so long as there is a prospect of continued progress the tactical research responsible must surely receive a substantial share of available funds. Nevertheless cancer research has so far had little effect on the outlook for patients suffering from most of the more common cancers, and it is this which calls, in addition, for the long term strategic approach and appropriate investment.

Leaving aside the actual scale, how might a levy system for strategic research work in practice? Within a single centre devoted to cancer research and dependent on grants and contracts it may be decided internally to allocate a certain share of facilities to strategic research of wider relevance. The proportion may vary considerably between centres. At grant level, applications for a predetermined share of cancer funds could be reserved and judged by a special agency, solely on the basis of scientific merit and without concern for the primary relevance to cancer. For government funding it is important that the contribution, however it is organised, should be deliberately set aside from any earmarked cancer budget and for that matter from other earmarked budgets, for cardiovascular disease and arthritis, for example. The levy, even if small, should be quantified and announced, and it should not be regarded as covered by the existing public expenditure on such things as university research.

The decisions about the strategic or tactical aspects of individual research proposals, and their inclusive or exclusive relevance to human cancer, will still have to be made, no doubt by committees of temporarily faceless peers. But I come back in the end to a rather idealistic view that a great deal of responsibility lies at the grass roots, namely with the individual conscience of the scientist who initiates a programme and the collective conscience of the group who constitute the centre or institute. And for those dependent on cancer funds, the framework of reference for this conscience should be the donor, be it the reluctant taxpayer, the major benefactor or the sad widow who subscribes her pound or dollar. ● □

'international news

Mediterranean poison fish forecast

from Alexander Dorozynski
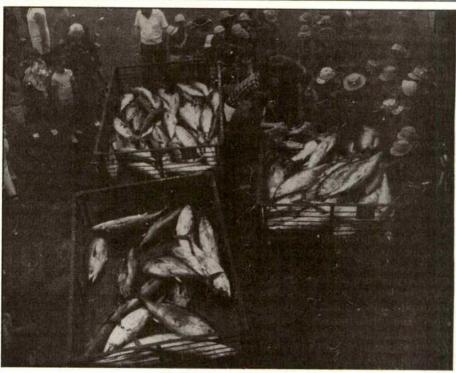
IF mercury contamination of Mediterranean fish continues at the present rate, it will take an average fisherman some 375 weeks, or 7 years, to absorb enough mercury for the first symptoms of poisoning to appear, and 1,000 weeks, or about 20 years, for poisoning to become lethal.

This startling forecast is made in a report prepared after the most extensive study of mercury absorption by the biomass yet carried out in the Mediterranean. Maurice Aubert, a medical doctor and director of the Centre d'Etudes et de Recherches de Biologie et d'Oceanographie Médicale (CERBOM) in Nice, has found that average mercury levels in 17 out of 31 species of fish studied exceed the maximum level considered as acceptable in France (0.5 p.p.m.). In nine species (including tuna, swordfish, crab, shrimp and red mullet) the average mercury levels exceed 1 p.p.m.

Dr Aubert points out that previous toxicological and epidemiological studies, notably in Japan and Norway, have shown that a total load of 80 mg of mercury absorbed by the organism of an average adult is lethal, and that the first neurological symptoms appear at about 30 mg. Fishermen along the Mediterranean are known to consume much more fish than other people—a weekly average of 2 kg of fish being a common figure.

Since it has been found that only 4 to 5% of mercury absorbed is fixed in the organism, a weekly absorption of food containing 2 mg of mercury will result in a fixation of 80 μg. At this rate, the lethal dose is reached in about 20 years.

In his report, prepared for the French Institut National de la Santé et de la Recherche Médicale (INSERM), Dr Aubert notes that his estimate is in agreement with pathological studies carried out in Japan among victims of 'Minamata disease'. Among the first victims were children, for whom the lethal dose was 25 μg of mercury. The fish in Minamata Bay contained about 10 p.p.m. of mercury, and the average

Mediterranean fish market : mercury at dangerous level?

diet of children included no more than 350 g of fish every week from the age of two until death at the age of five. This means that the total amount of mercury consumed was 3.5 mg a week, or a total of 525 mg during the three years. The accumulated lethal dose of 25 mg corresponds to a fixation rate of 5%.

Statistics concerning adult Japanese fishermen also support his estimates, he writes. From the moment a Minamata fisherman started eating fish containing 10 p.p.m. of mercury, it took about 300 days for the neurological symptoms to appear, and another 500 for the disease to reach the lethal stage. Given an equivalent consumption (and 10 times less contamination of Mediterranean fish), the process would take 10 times longer—somewhat more than seven years for the appearance of first symptoms, and twenty for the fatal outcome.

Dr Aubert, who is senior researcher at INSERM and whose studies are supported by the Ministry of Health, does not want to sound alarmist. A man of the sea and a well-above-average fish eater, he makes it a point to add that there is no reason to panic, to stop eating fish or to deprive fishermen of their livelihood. But there are good reasons for taking stock of the problem and finding ways of reducing mercury seepage into the sea.

Five years ago, Dr Aubert carried out an analysis of hair samples of four regular fish eaters in Nice; two of them were fishermen, one was Dr Aubert himself, and the other was his wife. In one fisherman, he found a total organic mercury concentration of 7.39 p.p.m. In Japan, first symptoms of Minamata disease were found to appear in people having accumulated in hair as little as twice this amount. Another fisherman had 1.58 p.p.m. and Mme Aubert, who is also a medical doctor and works with her husband, 2.88 p.p.m. As to Dr Aubert himself, his hair indicated a non-negligible level of 3.66 p.p.m.

It appears that this is the only study of mercury contamination of man that has been made along the Mediterranean. In Rome, several experts of the UN Food and Agriculture Organisation concerned with the Mediterranean were queried, and none was aware of any other similar study. Yet, the test is neither very difficult to carry out, nor costly.

It is known that the Mediterranean is particularly vulnerable to pollution. Warm and saline, almost tideless, with a limited continental shelf (an extended shelf favours a mixing effect) and communicating with the ocean only by a narrow strait, it is a relatively motionless sea. Most of its shores are densely

populated by people of different nations, some concerned with maintaining their rapid industrial expansion, others with achieving as rapidly as possible a degree of industrialisation equivalent to that of their neighbours.

Anti-pollution measures are not encouraged by energy shortage and economic crisis, and it is not unreasonable to believe that some aspects of pollution are ignored to avoid worrying consumers or restricting producers.

Mercury may be a case in point. Analysis of the mercury content of water is difficult and unreliable, but it is known that even if undetected amounts of this metal are present in water, it can be accumulated along the food chain, reaching particularly large concentrations in long lived and carnivorous fish, such as tuna.

Sources of mercury contamination are numerous and only a thorough investigation can identify them and attribute to each its share. From Barcelona to Genoa, some 50,000 industrial plants, large and small, add metals and chemicals to the sea. It has been estimated that the chemical industry alone is responsible for the rejection of 65 tons of mercury a year. Paper mills, electronic and pharmaceutical plants, and factories making explosives, add their share; so does agriculture, in countries where mercury-based fungicides are not banned. (In France alone, 4.5 tons of mercury are used in agriculture every year.)

Methyl mercury is the compound most readily absorbed by marine organisms. In Minamata Bay, methyl mercury was discharged by an acetaldehyde factory, but Dr Aubert notes that mercury salts can be methylated in the sea under favourable conditions, particularly in an acid environment. Thus other forms of pollution which contribute to a lowering of the *p*H of sea water catalyse mercury pollution.

The most typical example of this, he points out, is the result of the dumping at sea of huge amounts of residue by ships of the Montedison factory in Scarlino, on the Italian coast facing the island of Elba. The factory produces titanium dioxide, rejecting among other chemicals and metals, sulphuric acid compounds with a *p*H of 0.2, causing the lowering of the *p*H of sea water over large areas. The rejection of these "red muds" (so called because of the colouring action of iron) started "on an experimental basis" in 1972 and went on for two years before the president of Montedison and four administrators were condemned to suspended prison terms.

By then, more than 1 million tons of noxious residues had been dumped north of Cap Corse, disrupting the ecology of an area where some fish were found with almost incredibly high mercury contents (one dolphin had a level of 74 p.p.m. in its liver, 51 p.p.m. in its lungs and 14.6 p.p.m. in its muscles.)

Natural mercury contamination also takes place in the Mediterranean, where deposits are known to exist along the shores. Natural conditions can also contribute to the formation of methyl mercury; sediments, where the *p*H is lowered by metabolic activity of micro-organisms, can be conducive to the transformation of mercury salts into methyl mercury (this phenomenon was reproduced *in vitro* in CERBOM's aquaria.)

Undoubtedly many other mechanisms are involved, and they are far from being entirely understood; researchers wonder, for example, why is it that in some parts of the sea, where the mercury content is high, the amount of mercury accumulated in the biomass is relatively lower than in other areas, where mercury is hardly detectable.

André Jarrot, Minister of the Quality of Life, points out that the actual mercury levels in fish represent no danger whatever for the average French or European consumer, but announces nevertheless that some measures are being taken.

Already, there is a directive that can ban the setting up in sensitive areas of industrial plants producing chlorine by electrolysis using mercury electrodes. Fungicides containing methyl mercury are no longer authorised and, in the paper industry, the use of fungicides and biocides containing mercury in any form is forbidden. Mr Jarrot points out that a plan is already under way to reduce mercury pollution by 50% in two years and by 90% in five years.

If this is carried out, the French may be as successful in controlling mercury pollution as they seem to have been in controlling bacterial pollution along the beaches, which had reached an unsafe level a few years ago.

Since then, Dr Aubert and other researchers have intensively studied the behaviour of earth bacteria in water; Dr Aubert has identified antibiotic secretions in water and also shown that some marine micro-organisms secrete nucleoproteins that act as "telemediators" (which are inter-specific, but otherwise comparable to pheromones secreted by insects and animals) to trigger or interrupt antibiotic secretion in other micro-organisms. His studies of the antibiotic properties of the sea have led him to develop, in collaboration with Professor N. Desirotte, a mathematical formula to determine the point of discharge at sea of domestic sewage, whether treated or not. The equation is

$$\log C = \log (C_0/h) - (\beta\gamma/1.1)t$$

where C_0 is the bacterial concentration in the sewage before discharge and C the concentration at time t (h) after discharge from an outlet at a depth h (m); β is a coefficient which depends on flow and is unity for a discharge of $1 \text{ m}^3 \text{ s}^{-1}$; γ is a coefficient of the bactericidal action of sea water and is unity for the Mediterranean (comparative measurements in other seas show that γ can vary, so that it is important for it to be included in the mathematical model).

This formula has been used to determine the optimal position of sewage outfalls from large cities, sometimes requiring an outfall several hundred

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If contamination by mercury is successfully coped with, another serious problem is nevertheless likely to emerge in the years to come: thermal pollution, notably from nuclear power plants, which are particular wasteful of heat.

So far there is only one nuclear plant on the Mediterranean, at Vandellos, about 30 km south of Tarragona on the Spanish Costa Dorada.

The plant uses the French graphite-gas system, and was built with French technical assistance three years ago. It is cooled with sea water pumped from a depth of 13 m at the rate of $33,000 \text{ l s}^{-1}$ and rejected directly into the sea. As it enters the plant, the water is chlorinated to prevent shell incrustations in the pipes, and more than 300 tons of chlorine are thus emptied into the sea every year.

The temperature of the water as it leaves the plant is increased by 6 or 7 °C; the temperature of the surface water is raised by 3 °C over approximately 10 hectares and by 1 °C over several tens of hectares. In the summer, water leaving the plant has a temperature of more than 30 °C—too high for any known Mediterranean life form.

In August 1972, the American ERTS satellite took a photograph showing a large dark area covering several hundred hectares off Vandellos and believed to correspond to an impoverishment of plankton life. But surprisingly enough and in spite of protests of fishermen who have complained that surface fish have disappeared from within a radius of several kilometres from the plant, no serious study has been made of the ecological effects of this thermal and chemical pollution. Such a study could have served as a model for future nuclear plants that are planned along the Spanish coast and in Séte, Martigues and Port-la-Nouvelle on the French side of the Mediterranean.

metres and even several kilometres from the shore so that, whatever the meteorological conditions, the bacterial pollutants do not reach the shore alive.

Thus pollution on the beaches of the Côte d'Azur, which had reached an unpleasantly high level a few years ago, has considerably decreased. Similar improvements are being undertaken in other Mediterranean countries. ☐

New deal in French research?

from the staff of La Recherche

FOR the first time since he came to power, M Giscard d'Estaing has invited a select committee on scientific research to the Elysée Palace. The report at the conclusion of this meeting set out in detail the government's scientific policy; in particular, it stressed that "the development and stabilisation of the French research efforts is becoming increasingly important, and France should rate among the leading countries with comparable resources in both the volume and quality of its research . . .". For the first time for many years, the government has thus confirmed its intention to give scientific research some degree of priority.

This political assurance is important insofar as the financing of research has been growing more and more difficult, because recent increases in research budgets have usually been lower than the average rates of growth for general equipment, and barely compensated for inflation. Although the GNP criterion may not necessarily be adequate in determining the research effort of a country, it is nevertheless significant that the portion of the GNP devoted to research in France has been consistently decreasing since 1967, falling from 2.3% to 1.7% in the past even years. This continuous diminution of the intensity of the research effort, along with serious problems of employment in science, has worried a large section of the scientific community, and the problem was brought to the public eye by two candidates for the presidency of the Republic in May 1974. M Giscard d'Estaing is now attempting to set matters straight in the projected budget, and so to turn away from the policy which he initiated when he was Minister of Finance.

The involvement of the Elysée is without doubt important politically, since it will give a major trump card to those responsible for scientific policy when the next budget discussions are being held—especially to the Minister or Industry and Research, M d'Ornano.

A meeting of ministers at the end of April will decide the main points of the 1976 budget and it will then become clear whether research is really to be recognised as a priority, and whether the increase in the finance available next year will permit real growth. And now, after the political assurances as to the principle of increased research efforts, and the speculation on the growth of the research budget, it remains to be seen whether French scientific policy will have new life breathed into it.

The first decisions announced after the meeting with the President already provide some useful indicators. On the level of the organisation and construction of research policy, important decisions are of three types. First, provision is made for the formation of executive groups to activate research in certain ministries. Their function would essentially be to coordinate and initiate scientific projects in ministerial departments. On the financial level, the *enveloppe recherche* which includes a large portion of non-scientific research, would be redefined. It will no doubt be extended in the future so as to cover certain other projects, such as the big civil aviation programmes and telecommunications research. This would allow better coordination of scientific policy. Provision is also made for the creation of a basis for intervention in the programme of the Délégation Générale à la Recherche Scientifique et Technique (DGRST). This measure could no doubt be most important for it would, in particular, allow the DGRST to instigate interdisciplinary research projects on various subjects of great general interest.

The second series of measures announced concerns research personnel. It is first stated that science posts will be created "regularly and continuously". Measures will be taken to reabsorb the jobs of researchers without contracts, and to facilitate the mobility of staff, especially concentrating on better coherence of contracts and the devising of appropriate incentives.

The third type of decision aims to involve the scientist more in the responsibilities of scientific policy. It seems that there are divergent views on this. The government has in effect announced, on the one hand, that a committee presided over by M P. Aigrain (and including two academicians) would study reform of the Acadamy of Sciences and, on the other, that the role and composition of the Consultative Committee would be redefined. This choice can be interpreted as follows. A new, rejuvenated academy, would give the scientific community a forum where ideas could be aired, and a framework within which committees of experts could be formed to report on scientific problems. The Aigrain committee has already set to work to this end.

The Consultative Committee would be required to translate the wider socioeconomic needs of the whole country into the language of the practical objectives and means of scientific policy. On one matter the meeting with the President gave few precise details, that is, on policies relating to industrial research. Certainly, there is mention of the "economic assessment of the research effort" and of the importance of research to aid export growth, but it stays on the level of good intentions. Economic assessment of research was already on the cards in 1966, when the bill was passed creating the Agence Nationale de la Valorisation de la Recherche (ANVAR) on the model of the MRDC; it would be an exaggeration to say that no progress has been made on this problem in the past 10 years, but people have realised that the relation between science and innovation is far from being as simple as they thought.

It is on this problem of policy towards industrial research that the government document probably says least. A special committee under M R. Poignant will be handing in a report very soon on this question to the Minister for Industry and Research. ☐

Oceanography in Korea

THE government of South Korea has recently been expanding its interest in science and technology, and nowhere is the interest more visible than in oceanography. Two years ago the Korean Ocean Research and Development Institute (KORDI) was established to coordinate the development of the country's marine resources and collaborate on an international scale. By 1977 the new Science City in Daejon will be housing KORDI and 16 other institutes in the process of being established; for the present KORDI is based in Seoul.

The growth of the institute depends much on international goodwill. The United Nations Development Programme has agreed to provide $800,000 over three years, and bilateral assistance is being sought from Japan, European countries and the United States.

A staff of 63 is envisaged by 1977 and recruitment will be a major problem. Since many Koreans with the appropriate experience are living abroad, an extra effort has had to be made to attract them back; salaries are higher than those in comparable government departments, and modern housing is also being made available. ☐

Congress to vet NSF grants?

by Colin Norman, Washington

IN a move which is sending shivers of apprehension through the scientific community in the United States, the House of Representatives has decided that Congress should vet every research grant that the National Science Foundation (NSF) wants to award, and that it should have a chance to veto those considered to be a waste of taxpayers' money. A little-noted amendment designed to do just that was attached to an otherwise routine budget bill last week, in spite of an urgent plea by one Congressman that to pass the measure would constitute "an act of public and scientific irresponsibility".

Even if the amendment does not survive passage through the rest of the Congressional mill—it must be approved by the Senate before it becomes law, and its prospects there are far from certain—the fact that the House passed it provides ominous signs of growing political disenchantment with expenditures on some kinds of basic research. Put more bluntly, it is clear that some Congressmen have discovered that many research projects provide plump targets through which they can show the folks back home that they are doing their bit to hold down public expenditure.

The amendment simply states that the NSF must supply Congress each month with details of all the research projects it wants to support, and that Congress should have 30 days in which to exercise a veto over any grant that it considers unworthy. A resolution passed by either the House or the Senate would be sufficient to kill any such project.

Although the amendment does not suggest any criteria by which Congress should judge the scientific worth of research projects, it asks the NSF to provide "all facts, circumstances and considerations relating to or bearing upon the decision of the National Science Foundation to approve said grants, including to the maximum extent practicable the manner in which the national interest will be fostered by the approval of such grants". Since the NSF awards about 14,000 grants each year, the measure would clearly be an administrative nightmare if it ever reached the law books.

Why has the House suddenly turned sour on the NSF? Part of the reason is the widespread publicity accorded to some broadsides recently delivered by Senator William Proxmire against a handful of the NSF's research programmes, and another factor is that the NSF has been caught up in a bitter controversy over school textbooks. The whole business, in fact, provides an excellent example of the irrational manner in which many policy decisions are taken in the United States.

The NSF's troubles began a few weeks ago when Proxmire, an influential and entertaining man who is closely watched by the press, launched an attack on a few NSF social science programmes by the time-honoured tactic of firing off press releases holding up the research to ridicule. In the space of a couple of weeks, he poured scorn on a study on romantic love, a project entitled "Hitchhiking, a Viable Addition to a Multimodal Transportation System", a study of the "Social Behaviour of Alaskan Brown Bears", and a "Preliminary Investigation of a Special Impact of Television on Blacks".

Even without Proxmire's stamp, the press releases would have been guaranteed newspaper space, for the study on romantic love—which Proxmire termed the "boondoggle of the month"—was so juicy that it was carried by virtually every newspaper in the country, from the *New York Times* downwards. No matter that the study was designed to investigate why so many marriages end in divorce, which is not an irrelevant factor in family life in the United States, the attacks precipitated a spate of reports of supposedly trivial or useless research projects being supported by government funds. Particularly prominent was a list of projects with funny-sounding names which was broadcast by Paul Harvey, a radio commentator whose utterances are carried by scores of local stations.

Although many of the projects on Harvey's list date back to the early 1960s and have long since been terminated, the upshot of all the publicity was that members of Congress have been deluged with letters from their constituents complaining about wastage of government funds. Since constituents' mail provides an indication of voter sympathies, which few Congressmen can afford to ignore, the NSF suddenly found itself on the receiving end of a good deal of flak.

One example of a trivial research project, which was mentioned during the debate and which has attracted a good deal of publicity, is a $70,000 study of perspiration in Aborigines. Though that study has attracted much ridicule, it was in fact designed by the Department of Defense in the hope of finding a way to prevent soldiers from becoming dehydrated in tropical climates after many hours without water —a problem which does not seem to be encountered by Aborigines.

Such ill-informed attacks on the NSF's research programmes are by no means new, and they would probably have had little impact this year had it not been for another dispute about the foundation's activities. That dispute concerns a school science course developed in the 1960s by the Educational Development Centre in Cambridge Massachusetts, with NSF funding. Called "Man: a Course of Study" (MACOS), it is a collection of films

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IT is now a year since the European Space Agency (ESA) was to have subsumed the role of ESRO (the European Space Research Organisation) and what was left of that of the European Launcher Development Organisation (ELDO). It was then that ESRO's Director-General Dr Hocker, completed his term of office, making way for an appointment to the new post of Director-General of the ESA. In the event the two got inextricably twisted together and the situation become further confused by the French government's overall review of its science budget and the future of the Ariane launch rocket development which had been accepted as part of the 'European' package.

At last the situation is clarifying. The appointment of a permanent Director-General has been at the root of the whole impasse and it is now clear that the compromise candidate Roy Gibson has been accepted by the two rivals in sponsorship, the French and Germans. They have agreed to drop their respective national candidates and accept the Englishman, who has been doing the job on an acting basis and under considerable difficulties for the past year.

A specially summoned meeting of the space ministers of the 10 member countries, the European Space Conference, is convening in Brussels this week to confirm the appointment. Seven directors to complete the ESA top management will be announced at the same time. The organisation has been running with three directors short since last year.

It is expected that the same ministerial meeting will decide one way or another on the formula now put forward by the French for ESA participation in the operating costs of the French launch range of Kurou in Guiana from which the Ariane launching rocket is to fly. The switch to the French-designed rocket has also involved a charge of launch range. The previous Europa launcher based on the British Blue Streak was launched from Woomera, Australia where at one time the Australians were offering to pay all the operating costs. *Angela Croome*

booklets and exercises designed for use in teaching anthropology to 11-13 year olds. It includes studies of herring gulls, salmon and Netsilik Eskimos—a primitive and near-extinct Eskimo tribe living in North-western Canada. The course is now fully developed, and the NSF has been actively promoting its use in schools.

Like a good many other school courses and textbooks, MACOS has, however, generated a good deal of controversy, as a result of which John B. Conlan, a conservative Republican from Arizona, has been trying to persuade Congress to stop the NSF promoting it. Conlan objects particularly to the studies of the Netsilik Ekimos because, he says, "communal living, elimination of the weak and elderly in society, sexual permissiveness and promiscuity, violence and other revolting behaviour" are recurring themes in the studies. About 1,700 schools throughout the United States have, however, decided that MACOS is acceptable and are using the course.

Conlan tried to delete funds from the NSF's budget for promoting MACOS when the matter was being considered by the Science and Technology Committee, but the move failed. The Director of the NSF, Guyford Stever, subsequently announced, however, that he would defer all funding for MACOS pending a complete review of all the NSF's policies for promoting educational courses.

Not satisfied with that, Conlan took his grievances to the floor of the House last week, but failed narrowly—by a vote of 196 to 215—to delete the funds from the NSF's budget. During the course of the debate, Richard Ottinger, a Democrat from New York, suggested that the course simply describes the Netsilik culture in non-offensive terms.

Nevertheless, several members of Congress indicated that they had received many letters from constituents about MACOS, following press accounts of the dispute, and that factor clearly added to the NSF's troubles in the House. Together with the widespread publicity surrounding attacks on supposedly trivial programmes supported by the NSF, the dispute about MACOS indicated to some Congressmen that the NSF should be brought under more intensive scrutiny, and Bauman's amendment seemed to provide a means for doing just that. Consequently, the a m e n d m e n t was approved by 212 votes to 199.

Exactly how Congress should decide which of the NSF's programmes are in the national interest, what criteria should be applied to censorship of educational programmes, and which research could be dropped without hurting the progress of science, is, however, another matter. □

THIS footprint may be 250,000 years old: one of the oldest recorded prints of man. Pressed into volcanic ash, it was uncovered with some others in 1970 during the construction of a dam near Demirköprü in Turkey. It is now on display at the Museum of National history in Stockholm, as part of an exhibition about the origins and future of man.

The Museum acquired the print from Mr Thomas Barton, one of the engineers working on the project in Turkey when the print was uncovered. He did not, however, actually see it extracted as he was working some miles away at the time. He was given it later by men at the discovery site, who told him of its possible interest, and when he returned to Stockholm he brought it with him.

The placard explaining the exhibit states that the Turkish Metal Research Institute in Ankara determined the age of the ash and that the National Laboratory of Forensic Science in Stockholm has examined the print and agrees with the Turkish institute's findings. The forensic laboratory however, denies having tested the ash. According to a spokesman there, two members of staff were only asked to determine whether the imprint was actually of a foot or not. In their opinion it was. Neither has the Natural History Museum itself carried out any tests on the ash, as the equipment at its disposal cannot cope with the supposed youth of the sample. In the opinion of Professor Welin, of the museum's research department, any tests he could do would yield only a very approximate indication of the age of the ash. And there is no equipment elsewhere in Stockholm which could make a more accurate determination.

Apart from the Turkish test, then,

where has the 250,000 year old estimate come from?

The impression of the heel is deeper than that of the instep and the whole print is slightly blurred, suggesting it was made by someone running in the ash. The foot was pointing in the direction of the River Gediz. The theory advanced to fit these facts is that a man was caught in the midst of a volcanic eruption and ran for his life towards the river, imprinting the ash which, because of rain and heat, was baked into that form and shortly afterwards covered with lava. And Mr Barton says that there was volcanic activity in Turkey 250,000 years ago.

If this theory is correct, the print would belong to the Quaternary period. The Museum states that the print predates Cro-Magnon man, who came to Europe at the end of the last ice age. If this is true the runner would have lived during the Pleistocene period—possibly at the same time as Neanderthal man.

As the dating of the print—and even the circumstances of its discovery—have been based largely on hearsay, it would be a good idea to confirm the results of the Turkish test. There are, however, no plans to do this. Only the Turkish Embassy in Stockholm is showing interest on the grounds that Mr Barton did not obtain the necessary official permission to export an antiquity from the country. (Mr Barton insists that he acted within the law in bringing the print back.) During the course of its enquiries into this aspect of the affair the Turkish Foreign Ministry may of course discover whether the print is in fact an antiquity or not. But that seems a rather back-handed way of finding out the truth about what could be an important anthropological discovery.   *Wendy Barnaby*

THE problems of higher and specialist education have always been particularly difficult for Soviet educationalists, who must seek the best means of using potential talents for the good of the state, while not departing from the theoretical tenets of equal opportunity. During the half century of Soviet rule, a number of solutions have been proposed. At one stage the exceptionally gifted were expected to develop their talents in extracurricular time (traces of this remain in the mathematical "o l y m p i a d s" for schoolchildren), whereas under Khrushchev, the policy was one of what may broadly be called 'sandwich courses for all'—a coordination of education and work which led to considerable interruption of production and the exasperation of supervisors and managers. During the past few years, the Khrushchev policy has been quietly modified, adapted and legislated out of existence, and a return to more conventional policies introduced.
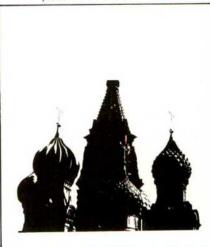
The new approach includes a certain amount of streaming, or rather creaming, of the highly gifted into specialist schools at a fairly early age. (One interesting facet of Soviet educational psychology is that, although it denies the existence of educational subnormality—except in the case of actual brain damage—it does admit the existence of above-average mentalities—although hedging its bets to the extent of claiming that the intelligence of a "lively" average child can be improved by educating him or her together with the highly gifted). Whatever the theoretical justification for creaming, current Soviet policy seems to have as its pragmatic aim the production of the vast work force of the intelligentsia needed for the expansion of the Soviet economy.

A recent *Pravda* article by the Minister of Higher and Specialist Secondary Education, V. Elyutin, outlines the plans for the 1980s and 1990s, and stresses the need for a new strategy in scientific and technological training for the next 10 years. Present plans include means of "making the training of specialists more flexible, and ensuring the organic connection of education and life, science and industrial activity". One defect of the Khrushchev system was that, for logistical reasons, young people were assigned to factory training that was unrelated to their talents or future professions. Another fault was the difficulty secondary school children had relating their school studies to the specialist technique of industry, in which they were supposed to participate. Minister Elyutin's stress on the cooperation of higher educational institutions (from Moscow Uni-versity downwards) with industry—a policy which is to be "extended further" in the immediate future—indicates that the emphasis will now be on such industrial training at a later age, when it can be more meaningful to the student and less disruptive to the factory. He notes, however, that such cooperation between institutes, or groups of institutes, and industrial enterprises may take "the most diverse" forms. The new plans are still production-orientated ("changes in specialities and specialisations" will be introduced

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Russia today

from Vera Rich, London

"in accordance with the needs of the national economy as they arise"), yet underlying the whole statement one senses a realisation that such a policy can only be fruitful if it is implemented in accordance with the special circumstances of each institute.

● The failure of the latest Soyuz to complete its link-up with the orbiting Salyut space station will not, repeat not, have any effect on the joint Soyuz-Apollo mission planned for July 1975. So we are told quite firmly by both parties to the project. Commander Richard Truly, the back-up capsule commander of the US team, said at a press conference in London, on his way to Moscow for a joint training mission, that the Americans still have every confidence in the joint mission and are satisfied with the safety aspects of the flight. The Russians, for their part, were eager to explain that this Soyuz was not the model which will be used for the joint mission, but an earlier version that had been "less diligently" checked than the system planned for the link-up. International goodwill and cooperation continues unaffected.

Yet in spite of the mutual reassurances, one wonders why the Soviet planners chose to announce the failure at all. It had not risen above the horizon of foreign tracking stations and need therefore never have been mentioned. Presumably it was the forthcoming joint mission which prompted the Soviet planners to make the announcement—but was their motivation one of cooperation, or fear of further setbacks? In the latter case, an aborted flight with the safe recovery of the cosmonauts might serve as a reassurance: even if the rocket fails, the men are safe. The old rumours of Vladimir Ilyushin, Gagarin's alleged predecessor on the launch pad, have never been entirely squashed in either East or West, and the Soyuz failure does offer a certain "substantiation", in that a crew-carrying rocket can be launched and fail without being tracked by foreign stations. The latest Soyuz venture is, at all events, a success for Russian safety procedures, and this fact may well have motivated the decision to release the news.

● Intimations that increasing pressure was to be exerted on participants in the illicit Sunday seminar for "refusnik" scientists have, unhappily, been justified by recent events. A letter to western "Academies of Science, Scientific Societies and Individual Scientists", signed by 45 participants of the seminar, states that increasing pressure is being exerted on individual participants "in order to interfere with the seminar's work." Methods mentioned include the issue of call-up papers for retraining in the Soviet Army (which could later be interpreted as access to classified information, a *prima facie* reason for refusing a visa), prosecution for "parasitism" (being without employment, although the scientists concerned have been deprived of their jobs as a result of applications for exit visas) and, in the case of Mark Azbel, the physicist, a kind of *de facto* exile. Professor Azbel, formerly Head of the Department of Electron Theory at the Landau Institute of Theoretical Physics of the Soviet Academy of Sciences, had been visiting relatives in Chernovtsy, and was about to return home to Moscow when he was stopped at the railway station and told not to go there for several months "or he would find himself further east" (in Siberia). Azbel is now stranded in Odessa without friends or money. It seems that, since the seminar continues to meet, in various venues, the authorities are now trying to erode it by pressure on the individual participants. They still seem unwilling to take the simple step, suggested by the seminar's founder, Aleksandr Voronel, of granting the whole group exit visas and thus letting them transfer their vexing activities to Jerusalem or Tel-Aviv.

news and views

Topography of ribosomal proteins

from a Correspondent

THE bacterial ribosome is the only cellular organelle for which a thorough understanding of both structure and function at the molecular level can be expected in the not too distant future. A central part of the structure–function correlation is obviously elucidation of the spatial arrangement of proteins within the ribosome; current interest in this aspect of ribosomes alone has produced a flood of well over a hundred papers in the last twelve months. Here I shall summarise briefly the present state of this problem of 'protein topography' with reference to the *Escherichia coli* ribosome.

In considering the three-dimensional arrangement of the proteins, a number of points must be borne in mind. First, the ribosomal proteins not only vary considerably in size, but, more important in this context, they may also have very irregular or elongated shapes within the ribosomal particle. Second, the proteins are not all present in equimolar amounts at all stages of the protein synthetic cycle; although this question of which proteins are 'unit' and which are 'fractional' still requires some clarification, it is certain that the ribosome cannot be considered a homogeneous entity. It remains to be seen whether the removal or addition of a fractional protein can seriously distort the arrangement of the other proteins. Third, conformational changes can occur as a result of interaction between the ribosomal sub-particles, or as a result of interaction with the various components of protein synthesis (tRNA, factors, and so on) and again it remains to be seen whether such changes are accompanied by a radical change in the protein arrangement within the particle. Fourth, only a small proportion of ribosomes are active in protein synthesis in *in vitro* systems, which raises the possibility that distortions of the protein arrangement may occur as a result of the isolation procedures used, or of course is a result of the procedures used to make the structural investigations. But since several techniques have been used during the last few years to examine the spatial arrangement of the ribosomal proteins, their success may be

judged by the measure of agreement between their various results.

The use of bifunctional protein cross-linking reagents is the most direct way of determining which pairs of proteins are neighbours within the ribosome. The chief problem here is in identifying the proteins contained in the cross-linked complexes; this identification can be made directly with antibodies specific to the individual proteins (for example Lutter, Bode, Kurland and Stöffler, *Molec. gen. Genet.*, **129**, 167; 1974). Most attention however has been given to the development of cleavable reagents, which enable the proteins in the cross-linked complex to be identified by gel electrophoresis, after chemical cleavage of the cross-link. The most widely used reagents for this purpose have been the *bis*-imido esters, and of these, *bis*-methyl suberimidate proved in earlier work to have the most useful properties (Bickle, Hershey and Traut, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1327; 1972). The reagent attaches to the ε-amino groups of lysine residues, and the cross-link is cleavable by ammonolysis.

Some authors, however, finding that the cleavage reaction did not proceed very satisfactorily, developed other reagents. The most noteworthy new reagents are methyl 4-mercaptobutyrimidate (Traut, Bollen, Sun, Hershey, Sundberg and Pierce, *Biochemistry*, **12**, 3266; 1973), and *bis*-azide compounds derived from tartaric acid (Lutter, Ortandel and Fasold, *FEBS Lett.*, **48**, 288; 1974). Both of these react with amino groups, but the former does not of itself introduce a protein–protein cross-link. Instead, the mercaptobutyrimidate effectively adds an SH group to the protein, and these SH groups can subsequently be cross-linked by mild oxidation. Cleavage of the isolated protein complexes is accomplished in the mercaptobutyrimidate case by reduction, and in the tartryl diazide case by periodate oxidation of the vicinal OH groups. Using this approach, many 30S protein pairs have been cross-linked, as have a smaller number of 50S protein pairs (Clegg and Hayes, *Eur. J. Biochem.*, **42**, 21; 1974). The possibility that some of the reagents

can themselves distort the protein arrangement remains, but the consistency of the results obtained with the different reagents argues against this. Further, in one case, a cross-linked protein pair (S5–S8) was incorporated back into a reconstituted 30S particle, without impairment of protein synthetic activity (Lutter and Kurland, *Nature new Biol.*, **243**, 15; 1973).

If the 30S ribosome is treated with nucleases under suitably mild conditions, it can be broken down into fragments of ribonucleoprotein which can be analysed for their protein and RNA content. Those proteins which are found together in such fragments are presumably neighbours in the intact particle, since it is unlikely that a ribonucleoprotein complex consisting of widely separated groups of proteins joined by an unprotected RNA strand could survive the nuclease treatment. Using this approach, the 30S particle can be readily split into two fragments of unequal size, the one containing eight to ten proteins and the other five or six (for example Morgan and Brimacombe, *Eur. J. Biochem.*, **37**, 472; 1973). Various smaller fragments have been isolated, containing sub-sets of these two groups of proteins, the smallest consisting of only two proteins.

A similar division of the 30S proteins into groups has been made from hydrolysates of a protein-deficient reconstitution intermediate (RI) particle (Zimmermann, Muto and Mackie, *J. molec. Biol.*, **86**, 433; 1974). In this type of approach, the proteins are of course not covalently attached to the RNA, and therefore special care must be taken to ensure that the observed results are genuinely specific. The importance of this point has been underlined by the finding that under conditions which promote 'unfolding' of the ribosomal sub-particles, the proteins lose their specific sites of attachment to the RNA (Newton, Rinke and Brimacombe, *FEBS Lett.*, **51**, 215; 1975). This almost certainly invalidates a number of previous fragmentation studies, particularly in the case of the 50S particle, although more recently some specific 50S fragments have been

described (for example Roth and Nierhaus, *J. molec. Biol.*, in the press). But in contrast to the 30S particle, the 50S cannot be split into two well-defined halves, despite the fact that the 23S RNA is readily cleavable into an 18S and a 13S RNA (for example Allet and Spahr, *Eur. J. Biochem.*, **19**, 250; 1971). The finding by neutron scattering that the centres of mass of protein and RNA are widely separated in the 50S particle as opposed to the 30S could explain this difference (Moore, Engelman and Schoenborn, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 172; 1974).

It has been known for some time that the 30S proteins interact with one another during *in vitro* reconstitution from protein and RNA in a very specific manner. These interactions have been incorporated into an 'assembly map' (see Held, Mizushima and Nomura, *J. biol. Chem.*, **248**, 5720; 1974, for the latest version). Although these interactions need not necessarily be a direct reflection of the protein neighbourhoods within the 30S particle, there has been striking agreement between those proteins which are found together in cross-linked pairs or ribonucleoprotein fragments and those which are related in the assembly map. It seems reasonable therefore to conclude that most if not all of the assembly interactions are indeed direct reflections of the ribosomal topography. The question of whether all the proteins in the complete particle have substantial contact with the RNA has yet to be settled, and it has been suggested that it may be more appropriate to think of the assembly interactions as being between regions of ribonucleoprotein as opposed to simply interactions between proteins (Kurland, *J. supramolec. Struct.*, **2**, 178; 1974).

There is of course as yet no corresponding assembly map of the 50S particle, since a successful total reconstitution of the *E. coli* 50S particle has only recently been achieved (Nierhaus and Dohme, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4713; 1974). Some assembly interactions have however been determined, using protein-deficient core particles as the starting point (Highland and Howard, *J. biol. Chem.*, **250**, 831; 1975).

Differential reactivity to chemical reagents has been used by many workers as a probe of ribosomal topography, the assumption being that those proteins which are most exposed on the ribosome surface will react most strongly with a particular protein reagent. Among the methods used have been digestion with trypsin, or reaction with kethoxal, various aldehydes and N-ethyl maleimide. Interpretation of the results of such experiments is however rather complex, since exposure or lack of exposure of a particular re-

active group is not necessarily a reflection of the protein topography in the wider sense. A reactive group on a protein could be shielded by RNA, or by the tertiary structure of the protein itself. Further, it is not easy to predict how far a chemical reagent can penetrate into the ribosome, and it is therefore not surprising that the degree of agreement between the various results is not very high. A good summary of the recent data can be found in a paper by Benkov and Delihas (*Biochem. biophys. Res. Commun.*, **60**, 901; 1974). At present, data from reactivity towards very large reagents are more easy to interpret, and two methods are noteworthy in this context. The first of these is measurement of the accessibilty to protein-specific antibodies (for example Stöffler *et al.*, *Molec. gen. Genet.*, **127**, 89; 1973), and the second is the iodination of ribosomal proteins catalysed by lactoperoxidase, of which a recent example is the work of Litman and Cantor (*Biochemistry*, **13**, 512; 1974). The general conclusion from all these methods is that all 30S proteins have some accessible groups on the ribosome surface, whereas the 50S proteins are not so readily accessible.

Several groups have attempted to coordinate the available data into three-dimensional models of the protein arrangement of the 30S particle. In all these models, the proteins have been represented as spheres, and the authors have been careful to point out that the arrangements are only schematic, and serve mainly as a means of testing the self-consistency of the various data. A technique has, however, recently been developed which casts doubt on the whole validity of even this crude type of model building: the direct visualisation of the proteins by electron microscopy of complexes formed between

ribosomal sub-particles and protein-specific immunoglobulins. This technique has become possible since both sub-particles have a readily recognisable shape in the electron microscope. Thus, if the ribosome is treated with a single protein-specific antibody, a ribosome–antibody–ribosome complex is formed, with the Fab arm of the antibody attached to a point on the ribosome surface. Several proteins have been localised on the surface of both sub-particles by this method (for example Tischendorf, Zeichhardt and Stöffler, *Molec. gen. Genet.*, **134**, 187; 1974), but the most important feature of the results is that while some proteins show a single surface binding site for their cognate antibodies, others seem to have multiple binding sites over a wide area of the surface, for example protein S4 (Lake, Pendergast, Kahan and Nomura, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4688; 1974). This indicates that the conformation of the proteins within the ribosomal particle is variable, and can be highly extended, which, as implied at the beginning of this article, alters the whole conception of the ribosomal topography, and rather changes the interpretation which must be made of many other data (such as the protein cross-linking results). In this context it should, however, be borne in mind that the overall dimensions of the 30S particle as measured by electron microscopy differ significantly from those estimated by low angle X-ray scattering in solution (Hill and Fessenden, *J. molec. Biol.*, **90**, 719; 1974).

In conclusion, the topography problem is still obviously some way from complete solution, but it is encouraging that the techniques in current use are by no means exhausted; further rapid progress can therefore be expected.

Devonian arthrodires

from B. G. Gardiner

A. RITCHIE'S article (see this issue of *Nature*, page 569) on Devonian arthrodires is a painstakingly compiled record of the distribution of the genus *Groenlandaspis*. He has shown that—like some other arthrodires (including *Bothriolepis, Holonema*) — *Groenlandaspis* is found in Devonian deposits all over the world and pleasingly has confirmed that the poorly known *Coccosteus disjectus* from Ireland and Bristol is yet another species of *Groenlandaspis*.

The distribution of *Groenlandaspis* will certainly excite palaeogeographic interest, but it is dangerous to jump to hasty conclusions about continental drift from such evidence

and Ritchie's caution is amply justified. As yet there is no articulated theory to enable us to deal with the zoogeography of Devonian fishes and so the significance of their distribution, which has been a puzzle for many years, remains an enigma. For most groups of fishes this will only be resolved when we have a clear picture of the configuration of the continents in the Devonian. But in the case of arthrodires, an entirely fossil group, an even more critical component is missing. For them we lack an acceptable phylogeny, a vital factor because palaeozoogeography can only make sense as the evolution of organisms in space and time.

The virus of acute diarrhoea
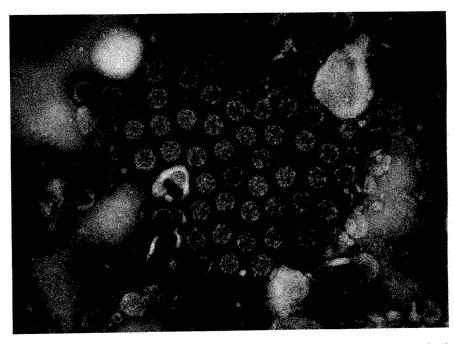
*from Anthea Thornton
and Arie J. Zuckerman*

ACUTE infectious gastroenteritis has long been one of the commonest causes of childhood illness throughout the world. In England and Wales (1961) acute diarrhoea accounted for 6% of deaths in the first year of childhood. But in technically underdeveloped countries, where malnutrition and other debilitating diseases are undoubtedly important contributing factors, deaths from acute diarrhoeal disease in early life are of such magnitude as to be a leading cause of mortality. Probably no more than an eighth of the infants and children of the world are free from appreciable risk of a lethal diarrhoea. Some episodes of gastroenteritis are clearly associated with certain bacterial pathogens; in at least 65% of cases, however, such agents cannot be isolated. Viruses of various type have occasionally been isolated but in most cases their pathogenic role has remained unestablished.

Recently a new candidate virus, originally classified as a member of the orbivirus group, was seen in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis (Bishop *et al.*, *Lancet*, **ii**, 1281–1283; 1973) and subsequently in negatively stained faecal extracts from such children (Flewett *et al.*, *Lancet*, **ii**, 1497; 1973). Morphologically similar particles have since been detected in duodenal fluids and faecal extracts by numerous other workers. Throughout the world, these viruses have now been reported in 396 out of a total of 827 (48%) of children with acute enteritis, and in only two of 357 children without infection. They have rarely been detected in children over the age of six, and are more often encountered in winter. There is now convincing evidence that the new virus is an aetiological agent in acute childhood gastroenteritis. The virus infects epithelial cells only during the symptomatic stage of the illness and its presence coincides with histological abnormalities and depressed duodenal mucosa disaccharidase levels. Seroconversion has been demonstrated in some children and oral transmission of infection has been achieved with one adult volunteer.

The virus differs from both the reovirus and orbivirus group in serology, morphology and polypeptide composition. The virions consist of a core about 38 nm in diameter, bounded by a membrane from which short cylindrical capsomeres radiate outwards like the spokes of a wheel. An outer layer of capsid subunits seems to be attached to the tops of the inner ones; they are

The characteristic morphological appearances of the 'rotavirus' in negatively stained faecal extract of an infant with acute gastroenteritis. × 126,000.

continuous at the periphery of the virions giving the impression of a continuous membrane surrounding the virus (intact diameter 60–65 nm). The wheel-like appearance of these viruses (see figure) prompted the suggestion that they be called 'rotaviruses' (Flewett *et al.*, *Lancet*, **ii**, 61–63; 1974), though Davidson *et al.* (*Lancet*, **i**, 242–246; 1975) now suggest that 'duoviruses' may be more appropriate.

Any new genus eventually proposed would probably also include the morphologically identical viruses that cause epidemic diarrhoea of infant mice (EDIM) and some outbreaks of neonatal calf and pig diarrhoea, the simian SA11 virus and the O agent isolated from gut washings in sheep and cattle. Complement fixation and immunofluorescent tests show that the human virus, the EDIM virus, and the Nebraska calf diarrhoea virus are also related antigenically. Immune electron microscopy revealed that human convalescent sera reacted with the human and calf viruses, both the intact particles and those which had lost their outer capsid layer, whereas calf antibody reacted only with the inner capsid layer of the human particles. Thus it seems that the inner capsid antigens are the common group antigens detected by complement fixation and immunofluorescent tests and the outer capsid antigens are probably type-specific. This view is reinforced by the finding that the human viruses do not infect calves.

The calf, pig and EDIM virus have been grown in tissue culture and lately the human virus has been shown to replicate in foetal intestine organ culture. Moreover immunofluorescent

studies showed that the virus-specific antigen was localised in the epithelial lining of the villi (Wyatt *et al.*, *J. infect. Dis.*, **130**, 523–528; 1974) which supports the earlier duodenal biopsy findings of Bishop *et al.* (1973). In the future the development of techniques by which human rotaviruses may be propagated to high titres in all cultures may eventually lead to development of a vaccine. An orally administered attenuated vaccine effective against the calf diarrhoea virus has already been prepared.

No other viruses have conclusively been shown to cause gastroenteritis in older children and adults. Parvoviruses (22 nm, DNA viruses) may cause bovine enteritis and similar viruses have been found by immune electron microscopy in stools from patients with gastroenteritis. They seem, however, to be found as easily in patients without gastroenteritis. Slightly larger (27 nm) picorna-like viruses have been detected in faecal extracts from an outbreak of gastroenteritis in Norwalk, Ohio. Transmission of infection and the detection of antibody to the Norwalk agent in infected people by immune electron microscopy have also been achieved. But the similarity of such small isometric particles to bacteriophages necessitates extreme caution in the interpretation of the findings in the electron microscope, and the specificity of virus-agglutinating reactions in immune electron microscopy studies has yet to be confirmed. In pigs and calves coronaviruses are also an important cause of enteritis though no cases have yet been reported implicating this group with human gastroenteritis.

Overall, existing evidence points to rotaviruses as a most important cause

of infantile gastroenteritis throughout the world. It is important to note, however, that to date few specimens have been examined from regions where gastroenteritis mortality rates are particularly high. The use of the electron microscope for rapid and reliable detection of rotaviruses in clinical specimens and the development of serological methods of identification will greatly facilitate further studies.

Does Hex A depend on Hex B?

from a Correspondent

THE question of the relationship of the N-acetyl hexosaminidases is still in dispute. It has been known for six years or so that two major electrophoretic forms of hexosaminidase (A and B) occur in human tissues and also that one of these (Hex A) is deficient in patients with Tay-Sachs disease, a fatal recessive disorder of infants characterised by the accumulation of a complex lipid, the ganglioside GM_2, the *in vivo* substrate of the enzyme, in the brain. The deficiency of both enzymes is also found in some patients —a variant which is often called Sandhoff's disease. It has generally been thought to be unlikely that two separate and independent gene loci code for the hexosaminidases, as the deficiency of both A and B in Sandhoff's disease would presumably require two separate mutations (at the locus for A and for B). The deficiency of Hex B alone has never been reported and Tay-Sachs disease is extremely rare—thus the coincidence of the two homozygous mutant genes in the same individuals would be expected to be very rare indeed. But twenty or so cases have in fact already been reported.

Biochemical and immunological evidence suggests that the enzymes are closely related, and two types of model have been proposed for their relationship. One is that Hex A and B possess similar and dissimilar subunits (for example $A = \alpha\beta$; $B = \beta\beta$). The other is that A and B are essentially the same protein, one enzyme being a secondarily (possibly enzymatically) modified form of the other.

In theory one might expect to learn something from the study of somatic cell hybrids. Human–rodent hybrid cells progressively lose their human chromosomes. By analysing the hybrids in a search for the coincident presence or absence of human enzymes and chromosomes genes can be assigned to particular chromosomes. When more than one gene (situated on more than one chromosome) is required to code for a particular protein or proteins the

number of independently located genes involved can be determined.

Information of this kind has been obtained for hexosaminidase but the results from different laboratories are conflicting (van Someren *et al., Humangenetik,* **18,** 171, 1974; Lalley *et al., Proc. natn. Acad. Sci. U.S.A.,* **71,** 1569, 1974; Nguyen Van Cong *et al., C.r. Acad. Sci. Paris.,* **278,** 1761, 1974; Gilbert *et al., Proc. natn. Acad. Sci. U.S.A.,* **72,** 263, 1975). In the most recent paper on the subject Gilbert *et al.* claim that there are indeed two independent gene loci that code for the hexosaminidases. The gene for Hex B is assigned to chromosome 5 and the gene for Hex A (in agreement with the other groups) is shown to be on the same chromosome as mannose phosphate isomerase(MPI), which has now been assigned to chromosome 15 (van Heyningen *et al., Ann. Hum. Genet.,* **38,** 295; 1975). This result differs from that obtained previously by Lalley *et al.* and Nguyen Van Cong *et al.* These authors never observed the retention of human Hex A in the absence of Hex B in the hybrid cells and suggested that Hex A requires the presence of two human genes for its expression and B requires only one of the two genes (which now seems to be on chromosome 5). These findings were consistent with both the favoured models for Hex A/Hex B relationship. The lack of observation of one particular combination is not however in itself very convincing evidence for a dependence model. The best supporting evidence would come from clones which have MPI but neither Hex A nor Hex B, predicted from this model for those clones which have chromosome 15 but not 5. Very few definite clones of this type have so far been reported. Gilbert *et al.* on the other hand claim to have clones of all the possible types (including Hex A+, Hex B−), which are consistent with their model. There are, however, some major problems that cast doubt on the interpretation of their results.

In order to avoid implicating two mutations in Sandhoff's disease, Gilbert *et al.* have suggested that the defect may be a mutation in a locus which controls the expression of both A and B or makes an activator. The first problem is that there is some evidence that certain Sandhoff patients at least make non-active antigenically cross reactive 'hexosaminidase' proteins (Srivastava and Beutler, *J. biol. Chem.,* **249,** 2054, 1974; Carroll and Robinson, *Biochem. J.,* **137,** 217, 1974). If the control model were right then no such protein should be synthesised. Second, another informative hexosaminidase variant has been reported in a healthy parent of a patient with Sandhoff's disease (Dreyfus *et al., New Eng. J. Med.,* **292,** 61,

1975). This individual has a deficiency of both Hex A and Hex B, as detected using an artificial substrate, and is probably heterozygous for the Sandhoff allele and one which produces kinetically abnormal A and B enzymes (which seem to hydrolyse the natural but not the artificial substrates for the enzyme). This variant strongly suggests that the enzymes must have polypeptides in common.

From the technical point of view, other workers have commented on difficulties involved in identification of the human hexosaminidase components in hybrid cells, and it may be that a problem of this kind is leading to misclassification of the enzymes.

So, the argument goes on . . .

Genes, enzymes and behaviour

from T. J. Crow

THAT heredity is a powerful determinant of behaviour was first cogently argued in Lange's book *Crime as Destiny* (Allen and Unwin, London, 1931). The controversy about the genetic contribution to intelligence has recently had a lively renaissance; H. J. Eysenck, a protagonist of the innate factor school, has also championed the view that there are important hereditary influences on the personality variables neuroticism and extraversion. In the more limited field of overt psychiatric disease the contribution of genetic factors to the major psychoses, schizophrenia and manic depressive illness, is now generally accepted and has recently been re emphasised by studies on adopted off spring of schizophrenic patients (for example Heston, *Science,* **167,** 249 1970; Kety, *Am. J. Psychiat.,* **131,** 957 1974). Evidence for genetic factors in neurotic illness (for example Shields *Monozygotic Twins;* Oxford Universit Press, 1962) is less well-known, but per haps equally significant.

Attempts to study the links betwee genes and behaviour have led to th development of animal models. I selective breeding experiments in rat Broadhurst (*Nature,* **184,** 1517; 195! was able to demonstrate a genetic in fluence determining 'emotionality', a defined by increased defaecation rate in an open field apparatus, and a independent factor of 'extroversio introversion' (*J. exp. Psychol.,* **56,** 34 1958).

Bovet and his colleagues (*Scienc* **163,** 139; 1969) demonstrated ve striking differences between strains, a consistencies within strain, in the rate and temporal patterns, of aquisition a conditioned avoidance response mice. Such experiments invite specul

tion concerning the neurochemical basis of the differences, and, in view of evidence for an important role for central monoamine neurones in behavioural control, focus interest on genetic variations in mechanisms regulating monoamine turnover

Recently Ciaranello, Lipsky and Axelrod (*Proc natn Acad Sci U S A*, **71**, 3006, 1974) have reported that the concentrations of three adrenal catecholamine synthesising enzymes, tyrosine hydroxylase, dopamine-β-hydroxylase and phenylethanolamine N-methyl transferase, are twice as high in the adrenals of Balb/cJ mice as in those of the related inbred strain Balb/cN, and that heterozygous progeny are intermediate between their parents in the concentrations of these enzymes Ciaranello *et al* (*J biol Chem*, **249**, 4528, 1974) examined the F_1, F_2 and backcross progeny of the mating between these sublines and concluded that single gene loci control the steady state concentrations of each enzyme The correlation between the activities of these enzymes suggested either that the loci were linked structural genes or that a single regulatory gene locus controls the phenotypic expression of the three enzymes Ciaranello *et al* (*Proc natn Acad Sci U S A*, **71**, 3006, 1974) also draw attention to differences between the two strains in fighting behaviour after a 2-week isolation period In this case analysis of the F_2 mice suggested the behavioural differences were determined by a single gene with fighting recessive

Such genetically determined behavioural differences could be coincidental, a cause, or a consequence, of the altered enzyme levels, but evidence for a relationship between aggressive behaviour and central catecholamines (Scheel-Kruger, Randrup, *Life Sci*, **6**, 1389, 1967, Welch and Welch, *Comnun Behav Biol*, **3**, 125, 1969) may be relevant to the association On the other hand much recent evidence supports the hypothesis that there is an association between central catecholamine-containing neurones and the mechanisms of reward (Crow, *Psychol Med*, **2**, 414, 1972, Ritter and Stein, *comp Physiol Psychol*, **85**, 443, 973) The question of whether unitary neurochemical mechanisms are associated with single dimensions in behaviour is one which increasingly will have to be approached

On the opposite side of the coin is an accumulation of evidence for environmental influences on central catecholamine turnover (Thierry, Javoy, Glownski and Kety, *J Pharmac exp Ther*, **63**, 163, 1968, Lewy and Seiden, *Science*, **175**, 454, 1972) Such findings may illuminate how genetic and environmental influences interact in the pathogenesis of the psychoses

A plasmid dissected

from David Sherratt

A NUMBER of features has made the bacterial plasmid Col E1 an increasingly attractive DNA molecule for studies of genetic organisation, DNA replication and gene expression Its small size of $4\,2 \times 10^6$ daltons, sufficient to code for about seven average-sized proteins, makes it easy to isolate and handle in analytical studies and its replication can conveniently be studied both *in vivo* and in a soluble *in vitro* system In addition, the Col E1 molecule has a single unique site for double strand cleavage by the sequence specific restriction endonuclease *eco*R1 This provides an invaluable positional reference for structural and replication studies of Col E1 and also provides an ideal tool for *in vitro* construction of hybrid DNA molecules containing DNA from either pro- or eukaryotes covalently linked to Col E1 (see for example Herschfield *et al*, *Proc natn Acad Sci U S A*, **71**, 3455–3459, 1974)

Localisation and analysis of specific regions of DNA (such as replication origins, strand interruptions, specific protein binding sites) by electron microscopy or other techniques requires some reference point In linear molecules with unique ends such as bacteriophage T7, the ends provide reference points (with ambiguity unless the ends are distinguishable) But Col E1 and many other DNA molecules of interest are either circular or like phage T4 have non-unique ends Various approaches have been used to provide visual markers in such molecules Inman (*J molec Biol*, **18**, 464–476, 1966) used mild denaturing conditions to visualise the AT-rich regions of phage lambda as 'loops' which occurred at fixed unique positions This technique has been widely used, though it is inapplicable for many molecules as small as Col E1 Another approach has been to construct heteroduplexes *in vitro* between molecules differing by a deletion or addition of genetic material (see Sharp *et al*, *J molec Biol*, **71**, 471–497, 1972), though this technique is not easily applicable to analysis of replicating molecules The use of sequence specific restriction nucleases such as *eco*R1 to introduce double strand breaks at specific sites in DNA is a relatively simple technique and is being extensively used in the physical mapping of small DNA molecules

Replicating Col E1 molecules have been isolated from exponentially growing *E coli* cells, by Lovett, Katz and Helinski (*Nature*, **251**, 337–340, 1974), from *E coli* minicells by Inselberg (*Proc natn Acad Sci U S A*, **71**, 2256–2259, 1974) and from a soluble *in*

vitro system which allows Col E1 initiation, semiconservative replication, termination and ligation to give complete closed circular duplexes (Tomizawa *et al*, *Proc natn Acad Sci U S A*, **71**, 802–806, 1403–1407, 2260–2264, 4935–4939, 1974) In each of these systems, the majority of replicating molecules when examined by electron microscopy are seen to be circular with two branch points, such *theta* structures are characteristic replicative intermediates of many circular replicons The positions of the branch points with respect to the *eco*R1 cleavage site were determined independ-

THE FATAL BALLOON ASCENT

THE readers of NATURE are no doubt aware of the fatal result of the recent ascent of the balloon *Zenith*, the following authentic details at first hand will no doubt be of interest —

CIRON (Indre), April 17

The *Zenith* was sent up on the 15th of April in order to determine the quantity of carbonic acid contained in the atmosphere at an altitude of 24,000 feet The "let go" was given at twenty-five minutes to twelve A M The captain was M Sivel, and there were only two passengers, M Gaston Tissandier and M Crocé-Spinelli The ascent took place gradually in a slight E N E wind, the sky being blue but vaporous The rate of ascent was calculated to be nine feet per second, but diminished gradually Shortly after one o'clock the altitude obtained was 22,800, and the passengers were quite well, although feeling weak The inhalation of oxygen produced good restorative effects when tried Then a consultation took place, and the *Zenith* being in equilibrium, a quantity of ballast was thrown over board M Tissandier then fainted, and is ignorant of what was felt by his friends

At eighteen minutes past two he was awakened by M Crocé-Spinelli warning him to throw over ballast as the balloon was fast descending He obeyed mechanically, and at the same time Crocé-Spinelli threw overboard the aspirator, weighing eighty pounds Tissandier then wrote in his book a few disconnected words, and again fell asleep for about an hour When he awoke, the balloon was descending at a terrific rate, no more ballast was left to be thrown away, and his two friends were suffocated Their faces had turned black, and the blood was flowing from their mouth and nose They were evidently dead It was a terrible situation

from *Nature*, **11**, 495, April 22, 1875

ently by each of the above groups of workers In each case, the distance from the cleavage site to the nearest branch (for molecules less than 60% replicated) was shown to be about 18% of the genome length, while the length from the other branch was variable and inversely proportional to the extent of replication of the molecule These results show that replication is initiated about 18% of a genome length from the *ecoRl* cleavage site and proceeds unidirectionally away from that site Whereas molecules in all stages of replication were observed *in vivo* and *in vitro*, molecules isolated from the *in vitro* system in the presence of glycerol (10%) and spermidine (2 mM) were nearly all about 7% replicated, indicating that there is a potential rate-limiting step at this stage of replication (*Proc natn Acad Sci USA*, **71**, 2260–2264, 1974)

Two other structural features of Col E1 molecules have also been examined with respect to the *ecoRl* cleavage site Col E1 molecules can be isolated as supercoiled 'DNA–protein relaxation complexes' which undergo a strand-specific single-strand cleavage after treatment with protein denaturing agents Helinski and co-workers (*Nature*, **251**, 337–340, 1974) first showed that the break in such molecules is located about 18% of the genome length from the *ecoRl* restriction site, that is, either at the origin/terminus of replication or at an equal distance the other side of the restriction site To distinguish between these possibilities Tomizawa *et al* (*Nature*, **253**, 652–654, 1975) tested the ability of pulse-labelled newly initiated Col E1 DNA to hybridise with Col E1 molecules in which the DNA in the region of the break in 'relaxation complex' had been removed but which still contained the sequences present in the symmetrical position 18% of a genome's length the other side of the *ecoRl* site The newly initiated DNA failed to hybridise to such molecules, showing that the break in 'relaxation complex' is coincident with the origin/terminus of replication and reinforcing the suggestion that relaxation complex is involved in Col E1 replication Col E1 supercoils containing a single stretch of RNA in either strand can be isolated after extensive replication in chloramphenicol (Blair *et al*, *Proc natn Acad Sci USA*, **69**, 2518–2522, 1972) Tomizawa and co-workers then went on to analyse the position of the gap created by removal of the RNA with respect to the *ecoRl* cleavage site and showed the RNA to be located at random, indicating that it is not a unique piece of RNA involved in initiation of DNA synthesis at the origin, though it could be involved in priming of 'Okazaki fragment' synthesis or

merely an artefact introduced during chloramphenicol replication

This type of experiment can be extended to analyse the organisation and control of Col E1 genes For example, the polarity of the 'nicked' strand in 'relaxation complex' with respect to the *ecoRl* site has been determined by Tomizawa (*Nature*, **253**, 652–654, 1975) and it is also known that Col E1 hybrid molecules with other DNA inserted at the *ecoRl* site fail to synthesise colicin yet remain immune to colicin killing (*Proc natn Acad Sci USA*, **71**, 3455–3459, 1974), suggesting that the immunity gene does not cross the *ecoRl* site, yet the colicin gene or one required for its synthesis does Using other restriction enzymes and an *in vitro* protein synthesising system it should be possible to map the Col E1 genes, isolate the gene products and study their regulation and functions

Quantising gravity?

from John Taylor

RECENT developments in unifying the forces of nature have been proving successful but so far have not involved gravity The quantum mechanical nature of the other three forces has up to the present prevented gravity from being treated alongside them due to great difficulties in setting up a sensible quantum theory of gravity Yet the problem of quantising gravity is a pressing one if a unified theory of all the forces of nature is to be obtained There is also the need for a quantised theory of gravity to describe the first 10^{-43} seconds of the big bang and to prevent the annihilation of matter at the centre of a black hole

These and other reasons have still left a great deal to be desired in the earlier programme of quantising gravity In the last three years great strides have finally been made by using techniques developed to handle the unified theories of weak and electromagnetic interactions This has proved possible since both unified models and gravity are invariant under gauge groups, these being internal symmetries in the former case and the space–time coordinate transformations in the latter The perturbation rules for calculating scattering matrix elements so as to be unitary at each order were developed in the 1960s for theories invariant under quite general gauge groups Unphysical modes of the gauge fields were found to be required to preserve unitarity, though these modes could never be created so never caused embarrassment

The recent advances have stemmed from attempts to obtain sensible answers for the resulting perturbation

calculations in the gravitational (The outstanding question has bee the removal of the ultraviolet di gences (arising from particles of a trary high energies in internal sta which plague any realistic quant field theory In the unified weak electromagnetic models these u violet divergences can be remo completely by absorption into and definition of the masses and charge the particles present the renorm ation programme Quantised gravil not explicitly renormalisable in sense that the ultraviolet diverge cannot automatically be removed redefinition of particle masses recent progress in quantum gravit in evaluation of some of these ul violet divergences and their remova additional terms (counter terms) in action function of the fields

The first step in this programme taken by 't Hooft and Veltman (*A Inst Henri Poincaré*, **20**, 69–94, 19 following the pioneering work of Witt (*Phys Rev*, **162**, 1195, 1239, 19 who evaluated for pure gravity counter terms that eliminate the ul violet divergences of all Feynman grams with a single loop of inter particles (in this case only gravito They showed that S-matrix eleme from such single-loop diagrams w finite after suitable field renorma ations had been performed, no infin counter terms were needed But such tricks were discovered wh eliminated single-loop diagram dive ences in the case of gravitation int acting with a scalar field, so that su terms were needed to be added to t action in such a way as to change considerably

This programme of investigating infinite counter terms are needed make single-loop S-matrix eleme finite has been extended to mo realistic forms of quantised matt fields interacting with quantised gravi Deser and Van Nieuwenhuizen (*Ph Rev*, **D10**, 401–420, 1974) have sho that extra counter terms are needed the case of otherwise free photons fermions, and a similar situation hol for charged mesons in self-interacti as well as gravitation, as it does photons are also involved (Nou Moghadam and Taylor, *Proc R So* in the press) This latter situation particularly appropriate since it allo for some of the subtleties of the unifi weak and electromagnetic interacti models

It could well be that the contributi of diagrams with higher numbers closed loops of internal particles cou cancel the infinities of the single lo ones However using the modifi action function, with single lo counter terms included, generates t new particles One of these is a scal

particle which can be made so massive as to put it beyond observation, the other, of spin two, destroys the theory since it is a ghost particle with a negative norm As such it violates unitarity, and certainly in particle physics ghosts should frighten one if they can be seen If this spin-two ghost particle could be decoupled from all other fields then it would have been rendered harmless, though recently it has been realised that such decoupling is very difficult for gravity in the presence of photons or scalar or spin-$\frac{1}{2}$ particles

If such is the case there are two ways forward in quantising gravity One is to start afresh from an alternative beginning to that of Einstein, this is also very difficult since there are almost unavoidable arguments which lead to Einstein's theory of gravity (Deser, *J gen Rel Grav*, **1**, 9–18, 1970) The other alternative is to investigate more carefully the forms of matter for which there is cancellation of the ultraviolet divergences from single loop diagrams This is a difficult but not impossible problem Its solution, if there is one, will give a hint as to the form of quantised matter–gravity interaction which is infinity free at all orders, it is to be hoped that there is only one such solution If there is no such form of theory then gravity will be different indeed from the other forces of nature

Alpha emission in fast pion reactions

from P E Hodgson

MANY researchers in recent years have provided evidence that nucleons in the region of the nuclear surface tend to condense into alpha particles This naturally affects the cross sections of reactions involving the transfer of a cluster to or from a nucleus, and it is by analysis of data on such reactions that an estimate of the alpha-clustering probability can be obtained

One of the most direct ways of demonstrating the existence of alpha particles on the nuclear surface is to knock them out with a suitable projectile Unfortunately the cross sections of these reactions are quite low so that thick targets must be used, and this stops most of the alpha particles from emerging Instead of trying to detect the alpha particles directly, the residual nuclei after the reaction had taken place were identified from their characteristic gamma-ray spectra It was then found that a substantial proportion of these nuclei were just the target nucleus less one, two or three alpha particles This strongly suggested that alpha particles were ejected from the nuclei by
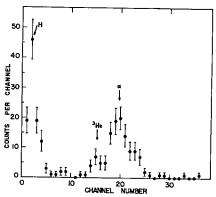
Fig. 1 Particle spectrum showing alpha particles and helions

the projectiles, though of course the possibility that the nucleons were ejected individually could not be excluded

This result was found in many experiments using fast and slow kaons and pions as projectiles (*Nature*, **249**, 616, 1974)

Some doubt was thrown on the interpretation of these results by an alpha particle knockout process when it was found by statistical model calculations that the observed distribution of final nuclei is what might be expected on purely energetic grounds It therefore became of great importance to demonstrate the reality of the alpha knockout process by detecting the emitted alpha particles directly

This has recently been done by a group at Saclay and Tel Aviv (*Phys Rev Lett*, **34**, 485, 1975) They bombarded ^{27}Al with 70 MeV negative pions and found a significant cross section for the emission of He ions, predominantly alpha particles They were able to overcome the problem of low yield by using the intense electron beam from the Saclay linear accelerator that produces a pion beam of intensity 10^6 pions per second This made it possible to produce a significant number of alpha particles in a target thin enough for most of them to escape

The observed particle spectrum at 90° to the beam is shown in Fig 1 and has a significant alpha peak and possibly a smaller peak due to helions The energy spectrum of the He ions in Fig
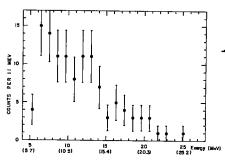
Fig 2 Energy spectrum of the He ions emitted from the bombardment of ^{27}Al by 70 MeV negative pions

2 shows that their intensity has a maximum at about 10 MeV, and falls to zero around 25 MeV The differential cross section for alpha-particle emission is 6 2 mbarn sr^{-1} in the energy range 5 5–30 MeV

Calculations within the framework of a cascade evaporation model indicate that such a yield of alpha particles can be explained by simple evaporation following the excitation of the nucleus by a series of pion–nucleon interactions Further work is now needed, particularly measurements in the forward direction to see if any of them can be attributed to a direct knockout of pre-existing alpha particles on the nuclear surface

Electric seaweed eggs

from Julian Lewis

MANY non-spherical embryos develop from spherical eggs What then is the origin of their asymmetry? In most cases the fertilised egg, despite its spherical shape, does not have spherical symmetry in its chemical constitution from the outset there is some local specialisation of the membrane or cortex, or an uneven distribution of material in the cytoplasm But there is no need in principle for an egg to start life lopsided It may have at first true spherical symmetry, and lose this through the development of an instability as it matures Its internal dynamics may be such as to magnify the slightest departure from symmetry into a full-blown polarisation The egg would thus spontaneously become polarised, but the direction of its polar axis would be subject to control by almost any sort of weak environmental vector

Jaffe and his colleagues have studied the eggs of the closely related seaweeds *Fucus* and *Pelvetia* (*Advances in Morphogenesis*, **7**, 295, 1968) These eggs, about 100 μm in diameter, are at first spherical Within a few hours after fertilisation they glue themselves to the sea bed, and then germinate they develop a bulge and become pear-shaped as the future rhizoid begins to grow out Practically anything, from a beam of light to a gradient of pH or a mechanical deformation, can govern the direction in which the rhizoid emerges This is strongly symptomatic of a symmetry-breaking instability, some other conceivable interpretations can be ruled out experimentally (Jaffe, *Science*, **123**, 1081, 1956)

Thus interest centres on the positive-feedback mechanism in the egg which amplifies and maintains whatever asymmetry happens to arise Jaffe suggests that a sort of self-electrophoresis

may occur if one part of the cell membrane transports ions at a different rate from the rest, a steady electric current may be driven through the cell, carrying towards the specialised patch of membrane just those charged molecules which support its specialisation This speculation has some experimental backing

In the first place, Jaffe (*Proc natn Acad Sci U S A*, **56**, 1102, 1966) aligned about 200 eggs, similarly polarised, in seawater in a loose-fitting capillary tube He found a potential difference of about 40 μV between its ends, and argued persuasively that this must arise from a flow of current, with an estimated density of 6 μA cm^{-2} at each rhizoid tip This might plausibly be driven by a cellular e m f of the order of millivolts

More recently, Nuccitelli and Jaffe have described in detail (*J Cell Biol*, **63**, 614, 1974) a probe small and sensitive enough to measure the electric fields and hence the current densities within 50 μm of a single egg (*Proc natn Acad Sci U S A*, **71**, 4855, 1974) The tip of the probe vibrates (at about 200 Hz), and picks up the minute change of potential (10^{-7} to 10^{-8}V) between the extreme points of its path, typically 30 μm apart The findings roughly match the results of the earlier work A striking phenomenon is the occurrence of large irregular spikes, lasting about a minute, during which the signal from the probe increases by a factor of ten or a hundred These spikes may well mark the insertion of fresh vesicles of membrane at the growing rhizoid tip, though sceptics might say they could be artefacts—transient diffusion potentials, perhaps, due to polyelectrolytes released from the egg and turbulently stirred about by the vibrating probe

Before the rhizoid axis becomes irreversibly determined at germination, there is an impressionable phase when environmental cues, such as light, can impose on the eggs a tentative and still reversible polarity Robinson and Jaffe (*Science,* **187**, 70, 1974) took a sieve with small round holes, plugged each hole with a *Pelvetia* egg at the impressionable stage, shone a light from one side, and measured the rates of entry and exit of radioactive calcium ions through the dark (prospective rhizoid) and light (prospective thallus) ends of the eggs The rate of entry on the dark side was at first about three times greater than on the light side, and the rate of exit about three times less The eggs, therefore, must pump calcium through themselves This pumping of calcium peters out towards the time of germinating, just as the flow of electricity begins.

One still does not know how strongly the ionic currents affect the cytoplasm,

or whether the cells are in fact polarised by self-electrophoresis But the evidence for persistent currents through fucoid eggs is important, if only in drawing attention to a factor in cell organisation which tends to be neglected, more because it is hard to measure than because it is insignificant, rare, or peculiar to seaweeds One might here perceive a distant echo of the finding, for instance, that the localisation of acetylcholine sensitivity in muscle cell membranes depends on electrical activity (Lømo and Rosenthal, *J Physiol, Lond*, **221**, 493, 1972)

Between Earth and Sun

from L J C Woolliscroft

A MIST (Magnetosphere, Ionosphere and Solar–Terrestrial Relations) Meeting, arranged by the Royal Astronomical Society, was held at the University of Exeter on March 25 and 26

THE significant role of atomic oxygen was stressed in a session on the lower ionosphere M R Bowman and L Thomas (Appleton Laboratory, Slough) gave two reasons for studying the density of oxygen first, it breaks the chain of reactions which starts with electrons and ends with negative ions, and thus it tends to prevent the loss of free electrons Second, it also breaks another chain by which positive ions such as N$_2^+$ react to form the series of water cluster ions, H$^+$ (H$_2$O)$_n$ where n can be at least as high as seven These cluster ions dominate the ion spectrum below about 85 km and it was encouraging to see that the model of Bowman and Thomas predicts an increase in atomic oxygen above this height with a diurnal variation below it P H G Dickenson (also Appleton Laboratory) presented new rocket measurements of atomic oxygen using the resonant fluorescence and absorption techniques These methods give better results than measuring the resistivity of thin silver films (oxidised by the atomic oxygen) because the response is faster His results seem broadly to agree with the Bowman and Thomas model but as yet the whole diurnal variation has not been studied

F N Byrne (Queen's University, Belfast) showed some preliminary data on the ionised and neutral concentrations of magnesium, aluminum and iron The ionised form of the metals has been shown to be important at altitudes above those characteristic of the cluster ions (typically 90–110 km) but the ratios of the ionised to neutral concentration is uncertain The reaction

of neutral metal atoms with nitric oxide ions to give metal ions should be very efficient and so some reverse mechanism is needed to explain Byrne's finding that significant densities of neutral atoms remain He suggested this was another role for atomic oxygen

The solar magnetic field and its effects are now felt to be more complicated W M Glencross (University College, London) challenged the conventional theory for the heating of the solar corona by shock waves He suggested that magnetic fields also play a part and showed Skylab and X-ray photographs to illustrate that the intersecting loop field configurations he proposes can in fact exist G M Simnett (Birmingham University) invokes the magnetic field at a few solar radii to account for the delays (at least 20 minutes) in the propagation of energetic protons and electrons from solar flares This process also accounts for modification of the energy spectrum of the particles during their passage through the corona which can be inferred from measurements near the Earth

The yields of turnips and other crops were discussed, together with the whole relationship between solar phenomena and the weather on Earth, by J W King (Appleton Laboratory) The correlation between various climatic and related phenomena, such as rainfall, surface pressure and temperature with the 11-year sunspot cycle is interesting enough, but what is much harder to envisage is why some properties correlate with the 22-year solar cycle of field reversals Perhaps the most striking of these are the periods of drought in the United States which at least in part led to the depression in the 1930s, and the mini-depression of the 1950s On a much shorter time scale the weather is altered by magnetic storms and after reversals of the magnetic field of the solar wind at the magnetosphere

These results were greeted with scepticism by a meteorologist in the audience who wanted details of a mechanism rather than King's qualitative suggestions of various possibilities including variations in the solar constant (the power emitted by the Sun), solar protons leading to water molecules in the atmosphere, and perturbations of the atmosphere at, say, 100 km causing a resonance in the lower atmosphere

D M Willis (Appleton Laboratory) presented results of how the magnetosphere might be involved in some form of coupling between the solar wind and the weather On the basis of the energy available it does not seem that the magnetosphere can provide the mechanism unless it is by some trigger-ing or resonance phenomenon ●

review article

Computer programming as a cognitive paradigm

P. J. Hayes*

As work on artificial intelligence has made increasingly clear, intelligent behaviour depends more on an organised knowledge of the real world than on problem-solving mechanisms This has led in artificial intelligence research to an increasing preoccupation with techniques for representing such knowledge, and recently to a view of programming as itself a form of knowledge representation.

As an essential part of the methodology of artificial intelligence research, the art and science of writing computer programs has inevitably been a central concern It has been suggested that artificial intelligence should be regarded as a science of which experiments are programs In the past few years, however, programming has become a focus of attention in its own right, and researchers have begun to look at the activity of writing programs as itself a problem area to which artificial intelligence might be applied, as it has already been applied to chess playing, recognising speech, or designing a bathroom Systems are now being designed and implemented to write programs to solve a given problem, which 'debug' programs[1], and which reason about the properties of programs Moreover, the development of the field since 1969 has been marked by a rash of new programming languages which represent a distinct break with the main computing tradition At the same time, both in Britain and in the USA, the main funding agencies (the Science Research Council and the Advanced Research Projects Agency), have noted the emergence of "automatic programming" as an important growth area in artificial intelligence

This apparently narcissistic concern with programming reflects a fundamental change in our view of what sort of knowledge has to be stored and used by an intelligent program, and how it can be represented The advance has emerged, characteristically, from a series of acrimonious debates, starting with a methodological dispute and finishing with a technical synthesis in which both sides can participate without losing face In this article I want to sort out a little of what is now agreed and what is not, and what consequences the new ideas have, both for our view of intelligence and for the development of programming To begin, however, we must be quite clear what we mean by 'a program'

Meaning and procedure

Asked to define 'program', most people would probably come up with something like 'sequence of instructions for a computer' This corresponds to the usual layman's view of the computer an electronic device which obeys long sequences of simple unambiguous instructions, very quickly, without errors But this definition would be adequate only for programs written in machine codes, which bear a direct relationship to the electronics which implement the instructions Most programs are written in much 'higher level', programming languages, such as ALGOL Programs in these languages are not simply sequences of instructions, but can have a complex syntactic structure More importantly, the meaning of a programming language is not

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directly related to the structure of any particular physical computer

The use of the term 'level' here refers to a fundamental computing idea An ALGOL programmer, for example, can ask for, say, a two-dimensional array of numbers When his program is run, complicated machine-code instructions will be generated which simulate this structure There are many ways of doing this which way is used is completely invisible to the ALGOL programmer It is a decision at a lower level than that of the ALGOL program A primitive operation (creating or using an array) at the higher level corresponds to an elaborate mechanism at the lower, the workings of which are invisible from above The ALGOL programmer may himself have in mind that his array represents the floor plan of a cathedral this, again, is an idea at a still higher level For, he could have used a wide variety of different data structures, (each probably with several ways of being implemented in code) to represent his conceptual structure Much of the art of computing consists in this use of complexity at one level to provide clean structures at a higher level, the meaning of which is independent of the encoding The development of large programs would be impossible without it

The meanings of high level programming languages have to be defined in terms of a 'conceptual machine' corresponding to the language This device may be quite unlike any physical machine for example, the 'ALGOL machine' is capable of directly evaluating arithmetic expressions, automatically maintains an internal dictionary of symbols in the program, and has a memory arranged as a stack (like a pile of plates) rather than as a sequence of boxes No physical computer was ever built with any of these characteristics It is important to realise that ALGOL programs are not merely abbreviations of machine code instruction sequences they have an existence quite independent of any physical machine The same ALGOL program can be implemented and run on a variety of quite different actual computers operating on totally dissimilar physical principles (for example, electronic, electromagnetic, fluidic) and yet the program retains its unique identity and meaning through these different incarnations It has been suggested[2] that a facility be incorporated in a high level language to allow a program to query the hardware of the machine about its input–output peripherals, so that it [the program] can organise its files appropriately for the machines it [the program] happens to find itself in from time to time

In some cases, the conceptual device underlying a programming language is not much like a machine at all The language SIMULA, for example, is designed to aid the writing of programs to simulate complicated system of activities, such as a dockyard To understand a SIMULA program one has to think of a collection of processes being created, running in parallel,

communicating with one another and altering their behaviour

The 'machine code' picture breaks down also, when we consider the relationship between high level programming languages and their meanings These languages do not consist of simple imperatives they also contain, for example, expressions which require to be evaluated, and declarations which define the meanings of names Some high level languages, indeed, contain no imperatives at all, although they un-ambiguously specify computations LISP, a language widely used in artificial intelligence work, is such a one In LISP, one defines functions (possibly recursively), and asks for them to be evaluated the language consists entirely of expressions

A program, then, need not be in any sense a sequence of instructions for a machine What all programming languages do have in common however, is a concern with activity, in the sense that they describe processes in some sort of device We will take this, then, as the definition of 'program' a program is a description of a process Any organised and constructive way of describing processes can be regarded as a programming language

Representing knowledge at the right level

Ever since the beginning of AI work, a central idea has been that in order to behave appropriately in some given situation, a program must have available, or be based on, knowledge of the situation and the circumstances To play chess, one needs to know a lot about chess-playing, to see a scene, one needs to know a lot about the sorts of object in the scene, about perspective, and about chiaroscuro

If this seems like a platitude, it is one It amounts to saying that there is no magic key to intelligence the science-fiction idea of the great mechanical brain suddenly becoming a super-intelligence when the last connection is soldered together, is a nonsense Paradoxically, the central theme of AI work diminishes the role of machinery, emphasising rather the importance of knowledge and inference (Platitude or not, however, this approach to understanding intelligence differs profoundly from that which would have us first understand how the brain works, in a neurophysiological sense)

Not surprisingly, then, many AI workers have been concerned with general ways of representing knowledge, with, we could say, general representational schemes For example, many people including myself have tried to adapt formal logical notations as vehicles for representing 'common-sense' knowledge of the everyday world others have emphasised, rather, the importance of such non-deductive modes of inference as the use of analogy and inductive generalisation For example, Newell has worked on a notation[3] for expressing observations of 'analogical similarity' such as "If you think of a nose as a snout, and feet as trotters, then a man can be thought of as a pig"

Merely having a notation to express knowledge is of no use, of course, unless the system can use the knowledge in some way Each scheme comes equipped with some processing rules which sanction certain inferences to be made using the notation Logical calculi have such rules of inference corresponding to logically valid deductions, schemes involving networks use, typically, a form of graph-matching to discover whether one network can be imbedded in another, Newell's analogy system uses an elaborate symbol-substitution mechanism to infer new, more complex, analogies from old ones, and so on

The information encoded in such a general scheme is at a higher level (in the sense described earlier) than that at which the actual program using the information is written (although such a program will almost certainly be written in a high-level language remember, there can be many levels) For example, the semantics of the knowledge encoded in the schemes—what it is about—is usually nothing whatever to do with the internal workings of any device, but rather concerns facts about some external environment chess for a chess-playing program, chiaroscuro for a vision program, mathematics for a theorem-proving program, actions and their effect on a world for a robot's planning program Moreover, one of these schemes

can be implemented differently in different programming languages without altering its meaning (such as by using different data structures to represent a network of relationships)

There have been, and still are, heated debates between different AI schools as to which are the most appropriate vehicles for expressing information about various task domains It is noticeable, however, that, in practice, no program which has actually achieved an interesting level of performance has ever used any of these methods to represent all the knowledge on which its performance depends

Many successful programs are simply one-off pieces of code in which the 'knowledge' is embodied directly in the way the details of the program are written For example (one of many), Kelley[4] wrote a program to find the outline of a person's head in a head-and-shoulders photograph (a police 'mug shot') This ingenious and successful program was based on a number of facts about heads and their appearance for example, that heads do not have long narrow projections, that they are more or less symmetrical, that they sit on shoulders, and so on But these facts were nowhere explicitly represented rather, the actual program was written in a way which was based on them For example, the no-projections fact was embodied in a heuristic rule by which the edge-following algorithm refused to follow such contours The knowledge was encoded at a lower level than that of an explicit representational scheme for expressing such facts

Even in programs which do use some such general scheme, one often finds that certain kinds of information, crucial to the success of the overall system, are embodied in the lower-level programming details An early example was Raphael's SIR[5], a program for answering simple English questions about a data base I will not describe its linguistic powers, which were rudimentary Its way of storing information and making inferences, however, was interesting as a pioneer attempt to represent relationships using a network

The fact "Hands have five fingers" was encoded by having an arc, from a node called HAND, to a node called FINGER, labelled with the relation HASASPART, and a note to the effect that there are 5 of them That people have two hands was encoded similarly If asked how many fingers people have, SIR could make the inference and reply, 'TEN' it did this by using a collection of little programs (written in LISP) which traced along arcs of the network This sounds quite elegant but notice that the inference requires also a general fact roughly, that if an X has N Ys as parts, and a Y has M Zs as parts, then an X has (M times N) Zs as parts And this fact is not in the network it is embodied in one of the little LISP programs

Another more recent example is provided by a much more sophisticated language-understanding system, Schank's MAR-GIE[6,7] Schank uses an intriguing graphical notation for expressing concepts and facts, which he calls 'conceptual dependency' notation Many claims are made for the generality of this notational scheme and yet, when one looks at the details of the system, one finds that the actual inferences are performed by specially-built LISP programs ("inference molecules" see ref 8) which directly encode most of the general facts which are needed

Special versus general

Now, what is wrong with having 'real-world' knowledge embodied directly in lower-level code in this way? If the program works satisfactorily, is that not enough? What more objective test could one have of a program's abilities? AI workers can be placed into two camps by the answers they give to such questions We could call these the specialists and the generalists

The former would tend to say there was nothing wrong with such encoding, that a program should be judged by its behaviour They have traditionally been concerned to get programs to perform well in limited task areas, using whatever means come to hand Joel Moses, author of a very successful program for symbolic integration, makes spirited defence

of this point of view[9] Generalists (of whom I am one), would argue, on the other hand, that having general schemes for representing knowledge is an essential step in capturing the adaptability of intelligent behaviour Purely on engineering grounds, it is very hard to alter information encoded at too low a level SIR, for example, had to be almost entirely rewritten in order to extend its repetoire of conceptual relationships by just one addition, and was indeed abandoned for precisely that reason Kelley makes similar remarks about his head-finding program

More profoundly, since learning would seem to consist largely, in the addition to, or alteration of, a program's knowledge-store, it is vital that this knowledge be represented in a form in which it can be easily altered and added to, if meaningful learning by programs is to take place Winston's concept-learning program, for example[10], which can be taught descriptions of simple assemblies of bricks (arches, towers, piles), by showing it examples and counterexamples, depends for its abilities on having a very clean representation of concepts (in a network, in fact, although that is not essential) which can be progressively altered by local increments

More fundamentally still, it seems to me that we should query whether knowledge encoded at such a low level is really present in the program at all That a certain mechanism succeeds in a task is not adequate evidence alone that it in any sense knows what it is doing A cabbage leaf channels rain to the root of the plant very efficiently but we would not say that the cabbage knew about rain

For several years in the mid-1960s, the 'generalist' and 'specialist' themes in AI research developed almost independently Some people worked on both sides of the methodological gulf at once but even then, there seemed to be very little generality visible in the *ad-hoc* specialist performance programs, and even those of the generalist persuasion were forced to the use of *ad-hoc* coding in order to get adequate efficiency in actual working systems Many attempts were made to combine a general scheme for representing knowledge with a general-purpose heuristic mechanism controlling the application of the processing rules But these always resulted in systems which, when applied to realistically difficult tasks, got lost in uncontrollably large search spaces This is the now famous 'combinatorial explosion' For several years, this uncomfortable situation was blamed on the 'weakness' of the general-purpose problem solvers It was generally considered that when these became more 'powerful', as a result of identifying ingenious general heuristic mechanisms, the situation would improve Great hopes were attached especially to the development of logical theorem-proving programs

Internal and external information

As is now, in retrospect, clear, this diagnosis was mistaken The fault lies not in the general heuristic mechanisms, but in the interface between those mechanisms and the knowledge represented in the formalisms The latter, consisting solely of information about the external task environment, gave no direct guidance to the mechanisms which processed the information And without such guidance, no mechanism could possibly be 'powerful' enough to make appropriate inferences

Consider, for example all the facts which an adult knows about people One of them is probably that people bleed if you cut them, another, that they can walk If you want to get somebody across a room, the second might be relevant, the first not but that fact is not about the world, but about how to think about the world

The point is similar to the platitude with which we began the magic mechanism must be replaced by appropriate knowledge But in this case, information is required not about the external task environment, but rather about the internal 'environment' of the scheme's processing rules In a word, the system must represent, and use, information about its own thought processes

And as we have seen, a description of a process can be

regarded as a program It is not surprising, therefore, that the first expressions of this insight took the form of advocating the use of a suitable programming language as a way of representing knowledge of the external environment This is the content of the term 'procedural representation of knowledge'[11-13]

It is important to emphasise at this point that we have not simply moved full circle, back to the specialist programs with their 'knowledge' rigidly encoded in conventional programs Rather, the proposal was to lift some programming ideas to the same level as that of the general representational schemes, in the hope of retaining much of the plasticity and clarity of the schemes already in use, but allowing the expression of information which controls the inferential activity These programming languages are very high level

The first programming language of this kind to be developed was MICROPLANNER[14], and a brief example will show how such control information can be encoded in it Consider the statement if a black cat crosses your path, you will be lucky Expressed in a logical notation, this would be something like

((Exists X) Black(X) and Cat(X) and Crosses(X,path(you)))
$$= > Lucky(you)$$

In MICROPLANNER, it can be expressed in many ways, depending on what you want to do with it For example

```
(CONSE (LUCKY? YOU)?)
  (PROG(X) (GOAL (BLACK?X))
          (GOAL (CAT?X))
          (GOAL (CROSSES?X PATH?YOU))
```

The brackets are an inheritance from LISP, but what this means is, if you want to prove that YOU are lucky, try proving that there is a thing which is black and a cat and crosses your path— in that order Now, in fact, this is probably a very inefficient way to establish this, as there are a lot more black things than things which cross your path A better version might be

```
(PROG (X) (GOAL (CROSSES? X PATH? YOU))
         (GOAL (CAT?X))
         (GOAL (BLACK?X))
```

More dramatically, it can also be expressed

```
(ANTE (CROSSES?X PATH?YOU)
  (IF (AND (GOAL (BLACK?X))
           (GOAL (CAT?X))
  (ASSERT (LUCKY? YOU)))
```

which means, whenever it is asserted that anything crosses your path, see if it is a black cat if so, assert that you are lucky This is quite a different way of using the same fact Notice, though that the level of these programs is similar to that of the logic the MICROPLANNER system can clearly be seen to be performing inferences MICROPLANNER is as different from LISP, as LISP is from machine code

Facilities of this sort were first used, to powerful effect, in Winograd's now well known program[11] which held an English conversation with a human user The demonstration was very dramatic, and since then this and other, similarly high level, programming languages have come into widespread use in AI work

A high level programming language is based, we have said, on a conceptual device whose activity it describes Now, MICROPLANNER's device is not much like a machine at all it is, rather, an idealised 'thinker' States of the device contain structured collections of assertions—the thinker's beliefs—and rules for manipulating them Transitions between states correspond to inferences Winograd's program used this directly to model its beliefs Successors to MICROPLANNER retain these essential outlines, usually with more highly structured states This gives a new view of computation as controlled inference, and of machines as essentially devices for storing assertions[15]

This differs radically from the conventional semantics of programming languages It is too soon to tell how far-reaching an effect it will have on our view of computing in general, but it seems to be unlikely to be negligible Hewitt, who designed MICROPLANNER, has developed, for example, a very general theory of computing based on the idea of an 'actor'[16]

The now widespread conception of a programming language

as a representation of knowledge explains the interest in programming as an activity, with which we began For, now, programming is expressing knowledge, debugging is learning, and reasoning about programs is thinking about knowledge —one could say, introspection Sussman's work[10] on automatic debugging of programs is a good example his system analyses, classifies and corrects various kinds of programming error in simplified MICROPLANNER programs, such as the achievement of one goal being undone by a later action (this is a 'protection violation bug') He regards this debugging as a model of a theory-formation process start with a simple theory and enrich it by looking closely at the places it does not work The program is the theory

There is by no means universal agreement on the best way of expressing process-control information It is no longer fashionable, indeed, to argue that a programming-language notation is essentially the best way to convey this information I am myself working on a more direct notation for expressing such knowledge But what is accepted now, by almost all AI workers, is that much of the information stored in an AI program has to be about what to think, as well as about what is true This distinction cuts across the arguments about deductive or analogical assertions mentioned earlier and it represents, I believe, a real advance in our understanding of thinking

In AI, a program might be said to be aware of a thing when it has a sufficient amount of knowledge of that thing explicitly

represented and available for making inferences In these terms, the 'procedural representation of knowledge' is a step towards the construction of programs which are aware of their own thought processes One could think of a worse definition of consciousness

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articles

Tectonic implications of Precambrian shear belts in western Greenland

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The occurrence of four Precambrian shear zones in western Greenland may point to the existence of a lithospheric plate during Proterozoic times The large scale, ductile, transcurrent movements can be interpreted as tectonically deeper expressions of major transcurrent faults and may represent the internal distortion of a Precambrian continental block

WHETHER the plate tectonic regime which characterised at least the later part of the Phanerozoic was of equal significance during Precambrian times is one of the outstanding geological problems of the present time One approach to this problem is to examine the nature and distribution of large scale Precambrian displacements, to establish whether they are compatible with the neotectonic plate model (or with a modified version of this model, such as that requiring extensive internal distortion of lithospheric plates[1]), or whether they represent an entirely different tectonic system The occurrence of four major shear belts, up to 40 km wide, in the Precambrian of western Greenland (Fig 1) suggests that intracontinental displacements were significant in late Archaean–early Proterozoic times The shear belts represent zones of mainly ductile, simple shear strain with dominantly transcurrent displacements of possibly

up to 200 km At higher tectonic levels and at the surface these shear belts would have been expressed as major transcurrent faults The characteristics of ductile zones of simple shear have already been described[2-4]

Nordre Strømfjord Shear Belt

This belt is situated in the central part of the Nagssugtoqidian mobile belt (Fig 1) and can be traced for about 150 km between the coast and the icecap The coastal half of the belt, which is the only part so far investigated in detail[5], consists of strongly deformed granulite facies gneisses for which a shear strain (γ) of 6 has been calculated The belt is 15 km wide in the coastal section and so this shear strain implies a displacement of at least 100 km across the belt, including the transitional zones on either side The shear strain has been calculated from the reorientation of earlier planar structures, the rotation of which shows the displacement to have been subhorizontal and sinistral, with a subvertical shear plane The orientation of planar structures across the belt varies in a manner consistent with a wedge-shaped profile for the belt, narrowing upwards, with a wedge angle close to 40°

Towards the north-east the shear belt is crossed by an amphibolite facies–granulite facies boundary which postdates the shear deformation The decrease in metamorphic grade
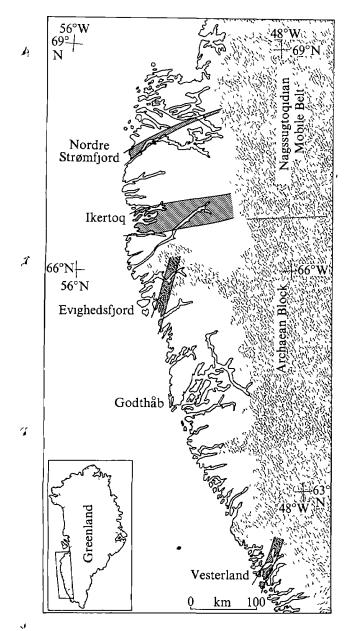
from the coast towards the icecap is accompanied by a decrease in width of the shear belt from 15 km to approximately 7 km, which is compensated for by an increase in shear strain, so that displacement across the belt remains almost constant regardless of width The eastward decrease in both width and metamorphic grade can be interpreted as the result of the differential uplift, by about 10 km, of the coastal region relative to the inland region (Fig 2) Numerous occurrences of pseudotachylite have been recorded in the Nordre Strømfjord region[6,7], but not within the shear belt

Ikertôq Shear Belt

This belt is 40 km wide, striking ENE with an exposed length of 150 km, and consists of highly deformed amphibolite facies gneisses bounded on both the northern and southern sides by granulite facies gneisses unaffected or only slightly affected by the shear movements[8,9] An elongate block of granulite facies gneiss with maximum width of 12 km occurs within the shear belt and is characterised by weak or absent shear deformation, we regard this block as a large augen structure A simplified map of the facies distribution in the coastal region is shown in Fig 3 The structural history of the shear belt is complicated in many places by later ductile overthrusting, but where these later effects are absent, as in Itivdleq Fjord, the original

characteristics of the shear belt are preserved In Itivdleq the intense L-S tectonite fabrics are consistent with a sub-vertical shear plane, with low angle stretching directions (x axis) corresponding to dextral transcurrent movements Estimates of the shear strain indicate values greater than $\gamma = 6$ which, if extrapolated to the full width of the Ikertôq Shear Belt, would correspond to a minimum displacement of over 150 km when allowance is made for the Kingaq and other smaller augen

The granulite facies rocks bounding the shear belt, and within the Kingaq augen, are structurally distinct from the amphibolite facies rocks of the shear belt in as much as tectonite fabrics related to the shear movements are either weak or absent, and large scale, open, fold interference patterns are present Discrete ductile shear zones, up to 50 m wide do occur within the granulite facies areas, however, and are characterised by retrogressive amphibolite facies assemblages These small shear zones are either subparallel or oblique to the regional shear direction, and have variable movement directions

Brittle movement zones, with pseudotachylite and pseudotachylite breccias, occur throughout the Ikertôq Shear Belt, but are most abundant within 5 km of both the northern and southern boundaries The strike directions of the zones of brittle movement are usually parallel to those of the gneiss fabrics although the directions of movement are mostly unknown The brittle movements extended over a long period of time, the earliest occurrences alternated with ductile movements while the rocks were still at metamorphic temperatures The latest pseudotachylites postdate dykes of Cambrian age
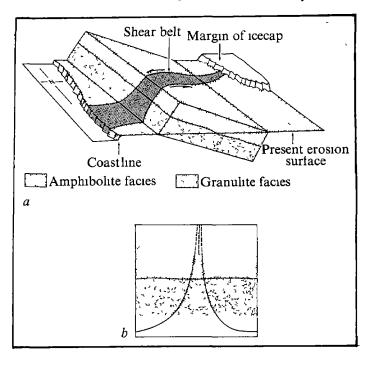
An important difference between the Ikertôq and Nordre Strømfjord shear belts is that in the Ikertôq Belt the mineral facies boundaries are very nearly coincident with deformation boundaries, whereas the mineral assemblage of the Nordre Strømfjord belt postdates the deformation, and metamorphic and deformation boundaries are apparently unrelated to one another

Evighedsfjord Shear Belt

This belt[10] has not yet been investigated in detail and its south-westerly continuation (Fig 1) is conjectural

In Evighedsfjord it is expressed as steep zones of highly

deformed, amphibolite facies gneisses retrogressively metamorphosed from surrounding, undeformed, granulite facies rocks The western margin is about 10 km wide granulite and amphibolite facies alternate in linear strips on a scale of tens or hundreds of metres The amount and direction of displacement have not been established, but smaller shear zones with similar trend and attitude are the result of sinistral transcurrent movement Pseudotachylite veins and breccias are abundant, and are concordant with the steep fabric of the highly deformed amphibolite facies rocks, although individual veins are transgressive Although they occur mostly in the amphibolite facies gneisses, there are some pseudotachylite breccias in granulite facies rocks otherwise unaffected by the shear movements The pseudotachylites postdate the ductile deformation but all of those seen predate the Kangâmiut dyke swarm[10], the emplacement of which can be shown to closely follow the ductile deformation

Vesterland Shear Belt

Although the Frederikshåb district in which this shear belt occurs has been systematically mapped on a scale of 1 20000 (ref 11) the shear belt is not known in detail as it was not recognised as such until the later stages of the mapping programme The belt has a NNE trend and can be traced over a distance of 60 km from Vesterland on the coast[12] to the icecap in amphibolite facies gneiss terrain[13] It is about 10 km wide and the marked tectonite fabric—and its orientation—are consistent with a subhorizontal shear direction Two domains of extremely high shear-strain occur at the margins of the belt Detailed analysis of the northern part of the shear[14] belt has shown the strain to be very variable, from $\gamma < 1$ to $\gamma \geqslant 6$, and, consequently, it has not yet been possible to integrate the strain across the belt to determine the amount of displacement, although it is clearly dextral

It is likely that other shear belts will be recognised in western Greenland in the future The possibility that a major shear belt exists in Godthåbsfjord has been suggested[14]

Age and seismicity of the shear belts

The limited radiometric data on rocks within and bordering the shear belts, and the ages of displacements relative to dyke swarms, show that movement on all four shear belts is likely to have been initiated 2,000–3,000 Myr ago, although there is no reason to suppose that all were initiated at the same time Movement along the northern margin of the Ikertôq Belt occurred as recently as the Mesozoic[15], and faulting of Jurassic dykes along NNE fractures close to, and within, the Vesterland Belt has been recognised[16,17]

The abundant occurrence of pseudotachylite in two of the shear belts, and its presence in a third, is a result of brittle deformation closely associated with the ductile shear deformation, although pseudotachylite formation also occurred during later movements The change from ductile to brittle behaviour and *vice versa* at this level in the crust is regarded primarily as a result of variation in strain rate rather than of short term changes in temperature or tectonic level We believe the formation of pseudotachylite by shear fracturing to be necessarily accompanied by the release of elastic strain sufficient to generate seismic waves (see refs 18 and 19), although the generation of seismic waves is not necessarily accompanied by pseudotachylite formation

This conclusion implies that two and possibly three of the shear belts described represented well defined linear seismic zones at the time of their formation, at the tectonic level now exposed At higher tectonic levels all four belts were likely to have been seismically active The depth represented by the tectonic level exposed at present is uncertain, but is thought to be 10–15 km below the contemporary surface, that is, at a depth similar to the deeper earthquake foci on the San Andreas[20] and Rhine Graben[21] systems at the present time We anticipate that the deeper, aseismic displacements on the San Andreas,

Rhine and similar fractures are represented by ductile shear zones comparable to the examples in Greenland The comparison with neotectonic, linear seismic belts can be taken a stage further in the case of the Nordre Strømfjord Shear Belt, the truly linear nature of which is indicated by its curving map outcrop, which is consistent with a small circle configuration on a sphere (We emphasise, however, that the geometrical identification of a small circle configuration does not necessarily imply any dynamic significance)
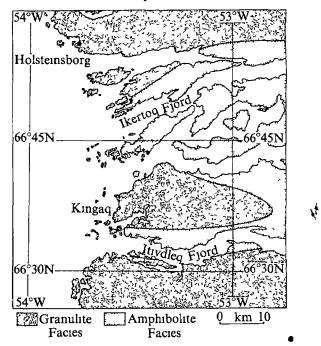
Implications for Proterozoic tectonics

The shear belts represent singular events which cannot be related to one another by the traditional orogenic concept A suggestion[22] that the Nagssugtoqidian boundary, with which the Ikertôq Belt is associated, may represent a plate collision boundary has not received subsequent support, although the evidence of ductile overthrusting on which it was based remains valid

It is difficult to envisage large scale transcurrent displacements of the type described without involving the existence of a horizontal layer along which the horizontal translation could be accommodated, the existence of such a decoupling zone defining the base of the lithosphere is, however, the *sine qua non* of the plate tectonic theory, although the evidence for the existence of such a layer derives almost entirely from investigation of activity at plate edges We suggest, therefore, that the continental mass within which the Precambrian shear belts were formed has the essential characteristic of a lithospheric plate, that is, a lower bounding surface which permitted differential translation relative to the underlying material The downward widening of the Nordre Strømfjord Shear Belt (Fig 2) if continued, may show how vertical and horizontal shear layers are connected

Discussion of the significance of the shear belts in terms of plate rigidity and plate margins is circumscribed by problems of definition Neotectonic plate boundaries are represented by discontinuities, although the Precambrian shear belts do not represent significant discontinuities at the tectonic level now exposed they would have done so at higher tectonic levels On one scale the shear belts represent non-rigid zones bounding more rigid crustal blocks, but on a larger scale they can be thought of as representing distortion within a larger continental mass The question of whether the shear belts could be

Fig. 3 Metamorphic facies distribution in the coastal section of the Ikertôq Shear Belt

Granulite Facies [▓]   Amphibolite Facies [ ]   0 km 10

regarded as plate boundaries cannot, therefore, be answered simply There is no doubt, however, that they represent displacements within a single continental mass and alternative conclusions can be based on this premise

Rigidity of lithospheric plates is only relative and the internal rigidity of neotectonic plates may have been overemphasised, although the evidence for the lack of overall distortion of shape[23] is very persuasive The Precambrian shear belts may be expressions of occurrences that are qualitatively comparable to the intraplate activity now represented by, for example, the East African Rift system, the Rhine Graben, the North Sea Graben, or the South Siberian Fault system The apparently more intense Precambrian intraplate activity could be explained in terms of the greater time period represented by the western Greenland shear belts, which is possibly longer than the Phanerozoic

Alternatively, if the shear belts represent significantly greater intraplate activity during the Precambrian (thus indicating a significantly less rigid lithosphere) this would be consistent with a relative mechanical weakness resulting from a greater width–thickness ratio of the Precambrian lithosphere This could be because either the lithosphere was thinner, or because there was a large Precambrian plate, or it could be caused by a combination of the two

If, as is often assumed, middle and early Precambrian times were characterised by higher heat flows, a corresponding reduction in the thickness of lithospheric plates would be expected insofar as the lithosphere—asthenosphere boundary is defined by an isotherm The consequent reduction in mechanical strength, and its effect on tectonic behaviour, could then be compared with the more fractured nature of neotectonic plates where these are thinnest as a result of higher heat flows adjacent to constructive boundaries[23] Variation of rock composition, and melting point, with depth is, however, likely

to complicate any direct relationship between thermal gradient and lithospheric thickness The palaeomagnetic evidence[24] for the existence of a Proterozoic supercontinent at least 15,500 km wide, and possibly 21,000 km wide, is sufficiently convincing for the size of the Precambrian plate to be regarded as an important factor when mechanical behaviour is considered If both thickness and size varied then the width–thickness ratio of the Precambrian plate would, perhaps, be 3–5 times greater than that of a typical neotectonic plate

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Groenlandaspis in Antarctica, Australia and Europe

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Groenlandaspis is a member of the Arthrodira, a group of Devonian armoured fishes In the past Groenlandaspis has been found only in eastern Greenland but now Alexander Ritchie has recognised at least six species from sites in Greenland, Europe, Australia and Antarctica His account demonstrates that these arthrodires enjoyed a very wide geographical distribution during the Upper Devonian and that the family can be traced back into the Middle and Lower Devonian

Groenlandaspis mirabilis Heintz, from the late Upper Devonian or earliest Carboniferous of eastern Greenland, is one of the last known members of the armoured arthrodiran fishes The original fossil material[1-3] is limited in quantity, disarticulated and fragmentary and there is insufficient to justify either a full description of the species or a reconstruction The specimens were collected mostly by Scandinavian expeditions to eastern Greenland in the 1920s and 1930s Although very poorly known, *Groenlandaspis* is generally accepted as a very late representative of a very important group of arthrodires (the arctolepids or dolichothoracids) which reached their peak in numbers and diversity much earlier in Lower and Middle Devonian times Until very recently *Groenlandaspis* had not been positively recorded from outside Greenland

As a result of field discoveries in Australia and Antarctica between 1968 and 1974, and finds made in existing European museum collections in early 1973, knowledge of the genus and its closest relatives has increased dramatically The family Groenlandaspididae is now known to have occurred on at least four continents and to range in time from Lower Devonian until the very end of the period and perhaps even into the Carboniferous The genus can now be recognised from material discovered in southern Victoria Land, Antarctica, half a world away from its original occurrence in Greenland At the time of writing at least six species of *Groenlandaspis*—several of them new to science—are known from the Upper Devonian of eastern Greenland, Ireland, England, south-eastern Australia, and Antarctica A new genus of the family Groenlandaspididae has been recovered from the Middle Devonian of western New South Wales, Australia and the family can be traced back into the Lower Devonian in the form of *Tiaraspis*, a small arthrodire from Germany[4]

Most of these occurrences are now represented by abundant, well-preserved material The discovery of *Groenlandaspis* on four widely scattered continents indicates a virtual worldwide distribution in the Upper Devonian and the genus may well prove of considerable value in intra and intercontinental correlation, supplementing the evidence already available from the antiarchs, *Bothriolepis* and *Remigolepis*, with which it is commonly, though not invariably associated

Geographical distribution

The sequence of events which led to the discovery and recognition of *Groenlandaspis* on four continents has been described elsewhere[5] Devonian fish remains from Antarctica were first discovered by members of Scott's last expedition in 1911–12 and were later described by Woodward[6] Material from other sites in southern Victoria Land, collected by Gunn and Warren[7] during the 1957–59 Commonwealth Trans-Antarctic Expedition, has been described by White[8] The very fragmentary material, apparently of Upper Devonian age, included arthrodires, antiarchs, palaeoniscids, crossopterygians and elasmobranchs

Two recent geological expeditions to the same area recovered more, and better preserved, Devonian fish material from many scattered localities[9,10] Preparation of this material has revealed that most of the remains of arthrodires from the Aztec Siltstone of southern Victoria Land can be attributed to one or more species of *Groenlandaspis*
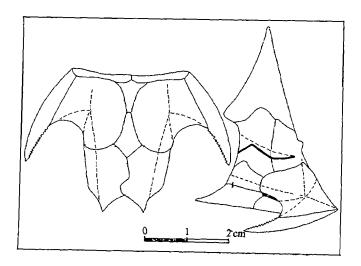
By a curious coincidence the same genus came to light in Australia while the Antarctic studies were in progress In May 1972 I investigated two Upper Devonian sites, 40 miles apart, in central New South Wales (NSW) and I recovered material representing two new and quite distinct species of *Groenlandaspis* from each locality The only previous record[11] of *Groenlandaspis* in Australia is probably incorrect although a member of the Groenlandaspididae is now known to occur in the same fauna, that of the Mulga Downs Group of Middle Devonian age

The new Upper Devonian records, both from the Hervey Group, come from the Cloghnan Shale in the Jemalong Range, some 20 miles west of Forbes, NSW and the Hunter Siltstone, Redcliff Mountin, 11 miles NNE of Grenfell, NSW Large quantities of fish-bearing material from these sites are under preparation and study in the Australian Museum In both cases *Groenlandaspis* is associated with *Bothriolepis* and *Remigolepis* spp but *Phyllolepis* is present at Jemalong and absent at Grenfell and the faunas are quite distinct in other respects

Between December 1972 and April 1973 I carried out a study tour of Devonian vertebrate collections in Europe and discovered two more species of *Groenlandaspis* in museum collections, the first records of the genus from Europe Both came from very late Upper Devonian deposits and from faunas which had been known as early as the 1850s

The first came from the Portishead Beds, exposed on the shores of the River Severn 8 miles west of Bristol in south-western England Small arthrodiran plates occur, tentatively

identified[12] as *Coccosteus? disjectus* Woodward, a spe recorded from the almost contemporaneous late Upper Dev ian Kiltorcan Beds in southern Ireland These small pl: from a collection housed in the Department of Geol University of Bristol, possess, however, the diagnostic fea of a species of *Groenlandaspis*

The second European discovery of *Groenlandaspis* cam light in the Sedgwick Museum, University of Cambri A small collection of unidentified, unlocalised and unregist fish plates again showed the distinctive and by now fam features of *Groenlandaspis* but of a species quite distinct fi the Bristol occurrence I also examined a small collection fish plates in the Royal Scottish Museum, Edinburgh, wh proved to be from the same source as the material from Sedgwick Museum Both had apparently come from a Upper Devonian horizon near Kiltorcan in southern Irel: a site better known for its abundant and well preserved pl fossils than for the associated fish fauna The material ci originally from the Geological Survey in Dublin, having b excavated in the 1850s and 1860s During a brief visit Dublin I established, from the original registers, that o 400 fish plates had been recovered from the Kiltorcan B between 1858 and 1861 (ref 13) and that shortly afterwa representative samples of the fossil flora and fauna had b: sent to Cork, Galway, Belfast, Dublin (Trinity Colle Edinburgh, Oxford, Cambridge and London Although so of this material has been lost over the years a surprising amol has now come to light as the result of recent enquiries

Some of the Kiltorcan arthrodiran plates were descril originally[14] by Woodward as *Coccosteus disjectus*, an identifi: tion generally accepted in stratigraphic accounts until recentl; Miles and Westoll[16] pointed out that *C disjectus* was not a va species of *Coccosteus* and that it may even have been an arc lepid and not a brachythoracid arthrodire Their suspici: were well founded, virtually all of the Kiltorcan arthrodir plates I studied belong to *Groenlandaspis* and can be placed one species—*G disjectus* (Woodward) There is evidence suggest the presence of a second species in the Irish material

Re-examination of existing collections in the Australi Museum has led to the discovery of a third occurrence *Groenlandaspis* in the Upper Devonian of NSW A figur specimen among some fragmentary fish plates from the Herv Range, north-west of Barkes in NSW, which was formerl; identified tentatively as a "crossopterygian dermal plate" c: now be recognised as a left anterior dorsolateral plate *Groenlandaspis* sp , three more plates of the genus have be discovered in the same collection, but there is insufficie material for specific identification The original site has n yet been relocated

Middle and Lower Devonian Groenlandaspidid:

All of the *Groenlandaspis* occurrences mentioned above : from undoubted Upper Devonian deposits It is now appare that *Tiaraspis* (Fig 1), a small arthrodire from the Lov Devonian of Germany[4] is an early, possibly ancestral, memb of the family The apparent absence of Middle Devoni representatives of the family initially presented a problem t that has now been solved following the recognition of a n: genus of the Groenlandaspididae in the Mulga Downs Group western NSW which has yielded large quantities of dissociat arthrodiran material

Wuttagoonaspis, a new arthrodiran genus[18], forms the bt of the fauna But the abundant associated material conta: many arthrodiran plates which cannot be assigned read: to a genus or species It is now apparent that many of 1 plates display features approximately intermediate betwe those of *Tiaraspis* and *Groenlandaspis* On other evidence 1 fish beds of the Mulga Downs Group are thought to be of ea Middle Devonian age so the new member of the Groenland: pididae present in the fauna, probably representing a n: genus, falls neatly into place

Fig. 1 *Tiaraspis subtilis* (Gross) Lower Devonian, Germany Trunk armour seen in ventral and right lateral view Overlap areas indicated by dotted line (After Gross 1962)

0   1   2cm
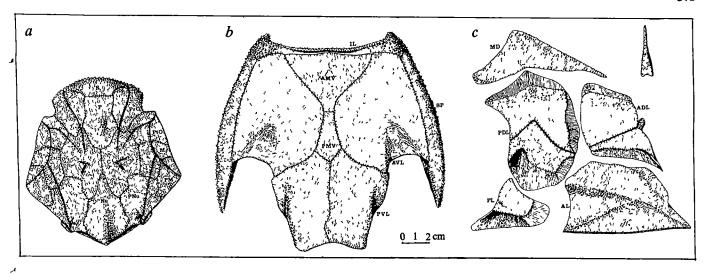
Fig 2 *Groenlandaspis antarcticus* sp nov, Upper Devonian, Aztec Siltstone, southern Victoria Land, Antarctica *a*, Headshield, *b*, ventral trunk shield, *c*, dorsal and right lateral trunk plates, shown detached All to same scale ADL,PDL, anterior and posterior dorsolaterals, AL,PL, anterior and posterior laterals, AMV,PMV, anterior and posterior median ventrals, AVL,PVL, anterior and posterior ventrolaterals, C, central, IL, interlateral, M, PM, marginal and postmarginal, MD, median dorsal, Nu, nuchal, P, pineal, PNu, paranuchal, PrO,PtO, pre and postorbitals, R, rostral, SP, spinal

Relationship between *Groenlandaspis* and the Groenlandaspididae

Although it is generally agreed that *Groenlandaspis* belongs in the family Arctolepida Heintz 1937 (=Dolichothoraci Stensio 1944) confusion exists concerning its relationships with other arctolepids, partly because there is inadequate material of the type species, *G mirabilis* Heintz

Denison[19] placed *Groenlandaspis* in the subfamily Phlyctaenaspinae whereas Obruchev[20] placed it in a family of its own —the Groenlandaspididae—and grouped it with the Holonematidae in a separate suborder, the Holonematoidei Miles[21,22] has shown that the Holonematidae are brachythoracid arthrodires at an advanced coccosteomorph level of organisation, although they are phylogenetically distinct from all other brachythoracids In spite of certain similarities between the Holonematidae and the Groenlandaspidae a close association seems unwarranted at present

I propose a classification which is a modified version of that of Miles[21] in which the name Arctolepida (which has precedence) is preferred over the name Dolichothoraci, and in which the Tiaraspididae are merged with the Groenlandaspididae

Suborder Arctolepida

Family 1, Actinolepididae, 2, Phlyctaenaspididae, 3, Groenlandaspididae, 4, Williamsaspididae, 5, Aggeraspididae
Amongst these the Groenlandaspididae are most closely related to the Phlyctaenaspididae on the evidence from the trunk armour

Family Groenlandaspididae Obruchev 1964

Obruchev[20] and Romer[23] included two poorly known genera, *Grazosteus* and *Tropidosteus*, in the Groenlandaspididae but Miles[22] has shown that these forms are of uncertain affinities and they are not here retained in the family At present only three genera are considered to be undoubted members of the Groenlandaspididae— *Groenlandaspis* (Upper Devonian), a new undescribed genus from Mulga Downs Group, western NSW (Middle Devonian), and *Tiaraspis* (Lower Devonian)
Groenlandaspididae Euarthrodira with unreduced lateral trunk shield, long contact between lateral and ventral trunk shield Pectoral fenestra of medium size Spinal plate large Median dorsal plate long, extremely narrow with high pointed laterally compressed dorsal ridge Anterior and posterior dorsolateral plates high, short Dorsolateral canal with angular, dorsally directed flexure over posterior dorsolateral plate Paired anteroventrals absent Craniothoracic articulation well developed, condyles situated rather close together Headshield subpentagonal, posterior margin convex or angular

Groenlandaspis

Type species *G mirabilis* Heintz 1932 Groenlandaspis Series, Upper Devonian (Famennian)-? Lower Carboniferous (Tournasian) Eastern Greenland

Discussion *G mirabilis* is a rather large arcolepid of which most of the plates of the trunk armour are known from isolated specimens and the headshield is known only from a natural mould of the posterior margin (and the articular fossae) and from an isolated central plate[1-3] From our present complete knowledge of the genus, based on the Antarctic material, it is clear that most of the Greenland specimens are identified correctly, except that the central plate figured by Stensio (ref 2, No 3, Fig 12) is the left, not the right, and should be reversed The posterior ventrolateral plate (PVL) figured by Miles (ref 3, Fig 9 *A-D*) at first sight seems to differ significantly from the PVL of *Groenlandaspis antarcticus* sp nov (Fig 2*b*) During examination of the original *G mirabilis* PVL in Copenhagen, it was found, however, that parts of two separate plates had been erroneously placed in close association, probably at the time of discovery in 1954 The posterior portion of the PVL, as restored, is incorrect and the shape almost certainly corresponded closely to that in *G antarcticus*

Although it would be difficult to distinguish between isolated or fragmentary plates of *Groenlandaspis antarcticus* sp nov and those of the type species, *G mirabilis* Heintz, there are sufficient minor differences to warrant separation in distinct species, at least until more complete material of the Greenland form becomes available The geographical separation alone suggest that it is unlikely that the Antarctic and Arctic faunas were conspecific

The genus *Groenlandaspis* can now be described in detail for the first time based on the excellent material from Antarctica, Australia and Europe Only the new Antarctic species is covered here but the diversity of the known species of the Groenlandaspididae is illustrated by a selection of the median dorsal plates of the new discoveries (Fig 3 *a-f*), the incompletely known MD of the type species, *G mirabilis* Heintz, is not shown here but seems to be close to that of *G antarcticus* sp nov (Fig 3*a*)

Groenlandaspis antarcticus sp nov (Fig 2 *a-c*)
Type the Australian Museum F54334, almost complete headshield, from Mount Ritchie, western side of Deception Glacier, south Victoria Land, Antarctica
Referred specimens AM F54336, F54314 (partial headshields), F56348 (complete ventral shield), F54337 (MD), F54382 (ADL), F54343, F54379 (PDLs), F54389 (AL), F54406, F56349 (PLs), F56222 (AMV), F55669 (PMV), F54344 (PVL), F56321-2 (IL)
Locality all from same locality and horizon as type, Mount Ritchie, Unit 62, Section A4 (24), with exception of F54314 (Portal Mountain), F56348 (Mount Metschel, southern end), F55669 (Section A2, small nunatak, north of Alligator Peak)
Horizon Upper Devonian, Aztec Siltstone, Taylor Group[24]
Description and discussion neither Woodward's material[6] nor that of White[8] from south Victoria Land contained specimens which can be positively attributed to *G antarcticus* sp nov The incomplete headshield of an aberrant arthrodire *Antarctaspis mcmurdoensis* White from Mount Crean differs strongly from the cranial material of *Groenlandaspis* collected by VUWAE 15 in 1970-1 The trunk plates figured by White (ref 8, Plate III, figs 1 and 2) as *Antarctolepis* show some groenlandaspid-like features but are quite distinct from the equivalent plates in *G antarcticus* There is some evidence for the presence of more than one species of *Groenlandaspis* in the Upper Devonian of Antarctica
The cranial shield, poorly known in the type species from Greenland, can be restored fully from the Antarctic discoveries An almost

complete specimen (AM F54334), recovered in many fragments from near the summit of Mount Ritchie, has been extracted and reconstructed Most of the remaining material has also been prepared

The restoration of the headshield of *G antarcticus* (Fig 2*a*) incorporates information from two other partial headshields and numerous fragments

The nuchal plate (Nu) is not particularly large, six-sided, bluntly pointed posteriorly and sharply pointed anteriorly The other median plates, the rostral and pineal (R, P), are separate, unlike in many arctolepids where they are apparently fused The pineal plate (P) is unusually large, extending posteriorly almost to the centre of the shield, completely separating the preorbital plates (PrO) and partly separating the large centrals (C) The latter are rather irregular in shape for an arctolepid, reflecting the extensive development of overlap areas to a degree more commonly found in brachythoracid arthrodires The articular fossae on the visceral surface of the paranuchal plates (PNu) are large, well developed and situated quite close together, as in *G mirabilis* The posterior margin of the PNu bears a prominent preglenoid process

The cranial sensory canal pattern is fairly conventional except for one feature not often found in arctolepids—the supraorbital canal extends beyond the preorbital plate (PrO) on to the central plate (C)

The trunk armour is known in considerable detail from many specimens of isolated plates (Fig 2*c*) and the ventral surface is restored (Fig 2*b*) from an especially fine, virtually complete specimen recovered from the southern end of Mount Metschel, Skelton Neve

The most distinctive plate of the *Groenlandaspis* trunk armour is undoubtedly the median dorsal (MD), an elongate, extremely narrow plate with a pointed dorsal crest and almost vertical lateral surfaces (Fig 2*c*) The ventral margin is shallowly indented for the insertion of the equally distinctive high but short, anterior and posterior dorsolateral plates (ADL, PDL) A unique feature in this genus is that the dorsal portions of the right and left PDLs meet one another in the middle, inside the very narrow base of the MD A broad rugose surface is developed over the area where they meet

A characteristic feature of the PDL in *Groenlandaspis*, already known from *G mirabilis* as shown by Stensio (ref 2, No 3, Fig 13), is the sharply flexed course of the dorsolateral canal crossing the plate The anterior and posterior portions of the canal meet almost at right angles This feature runs right through the family although it is not so extremely developed in *Tiaraspis* (Fig 1)

The anterior lateral plate (AL) is large and, for an arctolepid, relatively low and long The pectoral fenestra is closed behind by a smallish posterior lateral plate (PL) which is inserted dorsally into a deep groove in the PDL and attached ventrally to the dorsal lamina of the large posterior ventrolateral plate (PVL) On the ventral surface (Fig 2*b*) the most significant character is the absence of paired anteroventral plates which are present in the Actinolepididae The area is occupied by a large, semicircular anterior median ventral plate (AMV) which immediately suggests a close relationship between the Groenlandaspididae and the Phlyctaenaspididae[25]

Evolutionary trends

Through the known history of the Groenlandaspididae there is a noticeable trend towards progressive reduction in the height of the dorsal spine and a corresponding elongation of the median dorsal plate (MD) In the Lower Devonian *Tiaraspis* (Fig 1) the MD is short, very high and acutely pointed, in the new genus from the Middle Devonian Mulga Downs Group of western NSW (Fig 3*f*) the MD is slightly longer than high and less acutely pointed With the exception of the Grenfell form (Fig 3*e*) all of the Upper Devonian species of *Groenlandaspis* have an MD which is much longer than high (Fig 3 *a–d*), in all of them (Fig 3 *a–e*) the highest point is posteriorly placed and directed posterodorsally

A similar series can now be constructed for the other trunk plates, most usefully for the distinctive ADL and PDL plates, but there is often considerable variation within each fauna, the extent of which is still being analysed

Relative age of the known Groenlandaspididae

The three representatives of *Groenlandaspis* from the Northern Hemisphere (Greenland, Ireland and south-western England) are all known from the youngest Devonian rocks in their respective areas In Greenland the genus may last into the Tournasian The NSW specimens all occur in the Upper Devonian Hervey Group of central NSW (near Forbes, Grenfell and Parkes) but none of them come from the latest part of the Devonian sequence in their respective areas and it seems probable that they are somewhat older than the species from the Northern Hemisphere.
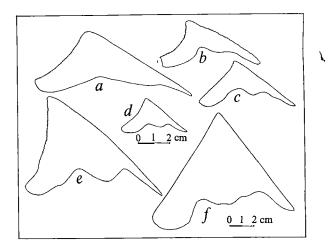
Fig. 3 Median dorsal plates of Groenlandaspididae in right lateral view *a–e*, All Upper Devonian and to same scale, *f*, Middle Devonian, slightly reduced scale *a*, *Groenlandaspis antarcticus* sp nov, southern Victoria Land, Antarctica, from Australian Museum F54337, *b*, *G disjectus* (Woodward) Kiltorcan, Ireland, Institute of Geological Sciences, London Zo3897, *c*, *Groenlandaspis* sp nov, Jemalong Range, Forbes, NSW, Australian Museum F56893, *d*, *Groenlandaspis* sp nov, Portishead, near Bristol, England, Bristol University Geology Department, BUGD 9926, 17850, *e*, *Groenlandaspis* sp nov, Grenfell, NSW, Australian Museum F56125, *f* gen et sp nov Mulga Downs Group, west of Cobar, NSW, Australian Museum F54567

Accurate dating and correlation of the specimens from NSW must await analysis of the rich associated faunas, but one unexpected feature is that the closest resemblances are not between those species of *Groenlandaspis* which are geographically close The Greenland and Antarctic species are alike in many respects as are the forms from Bristol, south-western England and Forbes, NSW But the Irish *G disjectus* and the distinctive new species from near Grenfell, NSW could not be confused with any of the other known occurrences

The value of the family for stratigraphic purposes has been demonstrated by the recognition of a groenlandaspid in association with a *Wuttagoonaspis* sp in the northern Dulcie Range, about 130 miles north-east of Alice Springs, Northern Territory, Australia (G Young, personal communication), a fauna which is clearly comparable to the Middle Devonian Mulga Downs Group of western NSW[18]

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Unique and repetitive sequences in multiple genes for feather keratin

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Embryonic chick feather keratins are a family of homologous polypeptide chains The mRNA coding for these has been obtained in a pure state and transcribed into complementary DNA (cDNA) using the reverse transcriptase from avian myeloblastosis virus Studies on the kinetics of hybridisation and reannealing of cDNA indicate that there are 25–35 different keratin mRNA species in the embryonic chick feather, and a total of 100–240 keratin genes in the chick genome Each keratin gene contains both a unique and a repetitive sequence It is proposed that the repetitive sequences are the keratin coding sequences and that the unique sequences correspond to untranslated regions

SINCE the original demonstration of highly repetitive, moderately repetitive and unique sequence classes in the nuclear DNA of higher organisms[1-3], it has been established that most highly repetitive sequences fall into a distinct class of simple-sequence non-coding DNA (satellites) occurring in long uninterrupted blocks in the genome[4,5] In contrast, the moderately repetitive DNA seems to be much more varied in function and organisation Much of the moderately repetitive DNA seems to occur in short segments intimately interspersed with unique sequences[1-3,6,7], and various models have attributed control functions to this class of DNA (refs 8–11) Whereas the structural genes for some (and presumably most) proteins occur in the unique DNA fraction[12-14], moderately repetitive structural genes have also been identified The genes for ribosomal RNA (r RNA) (refs 15 and 16), 5S RNA (ref 17), RNA (ref 18) and histones[19] are moderately repetitive and occur as tandem repeats in the genome Unique sequences are not, however, interspersed between these repetitive sequences and an example of a set of repetitive DNA sequences of known function, each directly linked to a unique sequence, as not previously been described

As chick feather keratins consist of a large family of homologous proteins[20-22], keratin genes must represent a family of non-identical moderately repetitive DNA sequences The availability of highly purified feather keratin mRNA (refs 23–25) affords an opportunity to study the organisation of this family of genes Feather keratin chains are the major protein products of the 14 day embryonic chick feather[26] About 20–25 distinct keratin chains, differing in primary structure by multiple amino acid substitutions, have been identified in the embryonic feather[20,21] Additional keratin chains are present in adult feather tissues[20] indicating at least 30 homologous keratin genes in the chick genome[22] These have presumably arisen by gene duplication and subsequent divergence

A 12S RNA species isolated from 14 day embryonic chick feather polysomes[23] was released from polysomes as an mRNP complex by EDTA treatment[24], bound selectivity to cellulose[23,24] and coded only for keratin chains in the wheat embryo cell-free system[25] Keratin mRNA migrated as one band of molecular weight 250,000 on formamide–acrylamide gels[24], indicating an untranslated sequence, including poly(A), of about 500 bases in addition to the keratin-coding sequence of 300 bases Since the keratin mRNA coded for at least four and probably all of the different keratin chains[25] is consists of a mixture of non-identical homologous keratin mRNAs of closely similar size

Keratin mRNA acted as an efficient template (ref 22 and D J Kemp, and G E Rogers, unpublished) for the oligo(dT)-dependent synthesis of complementary DNA (cDNA) by the DNA polymerase of avian myeloblastosis virus (AMV), as reported for other mRNAs (refs 27–29) Keratin cDNA migrated as a major peak (about 70% total) of apparent molecular weight 170,000 on formamide–acrylamide gels (D J Kemp and G E Rogers, unpublished), demonstrating that most keratin cDNA molecules must contain sequences complementary to at least parts of both the keratin-coding sequences and untranslated sequences I report hybridisation studies with keratin cDNA which demonstrate the presence of multiple keratin genes in the chick genome Each keratin gene contains interspersed unique and repetitive sequences

Hybridisation of keratin mRNA to keratin cDNA

The kinetics of hybridisation of cDNA to excess unlabelled mRNA are dependent on the sequence complexity of mRNA Comparison of the kinetics of hybridisation with those of a kinetic standard (for example, rabbit globin mRNA-cDNA, refs 30–32) allows an estimate of the complexity of keratin mRNA

As determined by resistance of the hybrids to the single-strand specific nuclease S1 (refs 33–35), rabbit globin mRNA hybridised to globin cDNA in a sharp transition (Fig 1a) with a mid point ($R_0t_{\frac{1}{2}}$) of 4×10^{-4} mol s l^{-1} (refs 30–32) Keratin mRNA hybridised to keratin cDNA in a broad transition (Fig 1a) with a $R_0t_{\frac{1}{2}}$ of about 1×10^{-2} mol s l^{-1} The kinetics cannot be ascribed to non-mRNA impurities in the preparations as they were reproducible using batches of keratin mRNA which was transcribed at efficiencies as high as 43% of the theoretical maximum[27-29] In addition, the keratin mRNA
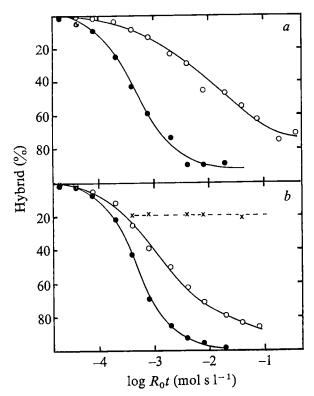
Fig. 1 Hybridisation of keratin and globin mRNA and poly(rA) to cDNA mRNAs were prepared from EDTA-treated polysomes as described[24] cDNA was synthesised in a system (50 µl) containing 50 mM Tris-HCl, pH 8 3, 8 mM dithiothreitol, 8 mM MgCl₂, 0 66 mM dATP, dGTP, dTTP, 0 1 mM ³H-dCTP (specific activity 26 2 mCi µmol⁻¹), 100 µg ml⁻¹ actinomycin D, 0 2–1 0 µg mRNA, 0 1 µg dT₁₂₋₁₈ and AMV–DNA polymerase, incubated for 1 h at 37°C, treated with 0 3 M NaOH and purified on Sephadex G-50 in 0 1 M NH₄HCO₃ (ref 28) The high molecular weight fraction (average about 170,000) of keratin cDNA, obtained by alkaline sucrose gradient sedimentation, was used for all experiments Hybridisations were at 60°C for 4 h in 0 18 M NaCl, 0 001 M EDTA, 0 01 M Tris-HCl, 0 05% SDS, pH 7 0 (NETS) The reaction mixtures (100 µl) contained 5,000 c p m keratin or globin cDNA (transcribed at 27 and 60% efficiency, respectively in the experiment shown) and varying amounts of mRNA For nuclease S1 assays, 10 µl aliquots were diluted into 150 µl of 0 03 M Na-acetate, 0 05 M NaCl, 0 001 M ZnSO₄, 5% glycerol, pH 4 6 (assay buffer) containing 12 µg of sonicated, heat-denatured calf thymus DNA, incubated with or without nuclease S1 (four units, purified through step four[35], at 45 °C for 30 min, TCA-precipitated and counted HAP assays (on aliquots of the same reaction mixture shown in (a) were as described[3] at 60 °C in 0 18 M Na-phosphate (Na⁺ molarity=0 18, pH 7 0) Where appropriate the results were normalised[6] to remove background and facilitate comparisons, the normalisation factor is shown in parenthesis for each curve, 0% indicates that a curve was not normalised *a*, Nuclease S1 assays ○, keratin mRNA–cDNA (0%), ●, globin mRNA–cDNA (5%) *b*, HAP assays ○, keratin mRNA–cDNA (10%), ●, globin mRNA–cDNA (20%), ×, poly(rA)–keratin cDNA (10%)

used here contained only one band on formamide gels and coded only for keratins in the wheat embryo system[24,25]

When hybridisation of keratin mRNA to cDNA was assayed on hydroxyapatite (HAP), a major transition was observed (Fig 1*b*) with a $R_0t_{\frac{1}{2}}$ of 9×10^{-4} mol s l⁻¹, about ten times faster than the same reaction assayed with nuclease S1 In contrast, the kinetics of hydridisation of globin mRNA–cDNA were identical whether assayed on HAP (Fig 1*b*) or with nuclease S1 (Fig 1*a*) The major transition of keratin mRNA cannot be explained by interaction of cDNA with the poly(A) segment of keratin mRNA as only about 20% of keratin cDNA molecules formed hybrids with poly(rA) (Fig 1*b*), and was not caused by self-complementarity of the cDNA since incubation of cDNA in the absence of mRNA for 0–4 h resulted in 10% binding to HAP
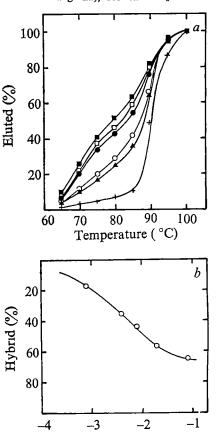
The thermal stabilities of keratin mRNA–cDNA hybrids changed as a function of R_0t (Fig 2*a*) At least two com-

ponents with T_m of about 72° and 90° were evident Hybrids which eluted from HAP at greater than 80° formed with a $R_0t_{\frac{1}{2}}$ of about $6-7 \times 10^{-3}$ mol s l⁻¹ (Fig 2*b*), a value similar to the rate observed using nuclease S1 (Fig 1*a*) In contrast, only one major component of high thermal stability was present in globin mRNA–cDNA hybrids (Fig 2*a*), demonstrating that the low thermal stability of keratin hybrids was not caused by their degradation under the incubation conditions used here

Reannealing of keratin cDNA with chick erythrocyte nuclear DNA

The reiteration frequency of sequences in cDNA preparations can be estimated by annealing cDNA with a vast excess of chromosomal DNA[13,14,36-38] As determined by nuclease S1 assays in low salt buffer (Fig 3*a*) chick DNA (labelled either with ¹²⁵I *in vitro*[39] or with ³²P *in vivo*) reannealed with a midpoint $(C_0t_{\frac{1}{2}})$ of about 1×10^3 mol s l⁻¹ (ref 38) Keratin cDNA reannealed to a vast excess of chick DNA with a $C_0t_{\frac{1}{2}}$ of about 4×10^2 mol s l⁻¹ (Fig 3*a*) As determined by nuclease S1 assays in high salt buffer (Fig 3*b*) ¹²⁵I-labelled chick DNA reannealed at a similar rate to that determined in the low salt assays but seemingly to a greater extent Assayed under these conditions keratin cDNA gave a broad curve (Fig 3*b*) suggesting two transitions with $C_0t_{\frac{1}{2}}$ values of about 4×10^1 and 1×10^3 mol s l⁻¹, respectively

Fig 2 Thermal stabilities of mRNA–cDNA hybrids Aliquots as described in Fig. 1 were diluted into 0 18 M Na-phosphate (pH 7 0) containing 100 µg partially re-associated calf thymus DNA and loaded on to HAP at 60 °C Hybrids which could not be eluted at 60 °C were melted by raising the temperature in 5 °C increments Two 2 5 ml washes (0 18 M Na-phosphate) were collected at each temperature and TCA-precipitated *a*, ○ Keratin mRNA–cDNA hybrids, after incubation to R_0t values (mol s l⁻¹) of ■, 8×10^{-4}, □, 4×10^{-3}, ●, 8×10^{-3}, ○, 2×10^{-2}, ▲, 8×10^{-2}, +, globin mRNA–cDNA hybrids, 8×10^{-4} *b*, Rate of formation of keratin mRNA–cDNA hybrids eluting from HAP at greater than 80 °C The normalised percentage cDNA bound to HAP at 60 °C (data from Fig 1*b*) was multiplied by the percentage of this bound cDNA which eluted at greater than 80 °C (data from Fig 2*a*), for each R_0t value shown

When assayed on HAP (Fig 3c) chick DNA reannealed with a major transition at a $C_0t_{\frac{1}{2}}$ of about 4×10^2 mol s l⁻¹, a value compatible with estimates of the size of the chick genome[3,14,40,41] Keratin cDNA gave two distinct transitions (Fig 3c) with $C_0t_{\frac{1}{2}}$ values of 7 mol s l⁻¹ (about 60% total) and 5×10^2 mol s l⁻¹ (about 30% total)

The thermal stabilities of the keratin cDNA–DNA duplexes (Fig 4a) changed with C_0t in a manner similar to but more marked than the mRNA–cDNA hybrids (Fig 2a) Two components with T_m of about 73° and 93° were evident Duplexes which eluted from HAP at greater than 85° (Fig 4b) formed with a $C_0t_{\frac{1}{2}}$ of about 6×10^2 mol s l⁻¹ From the data in Figs 3b, 4a and b it can be calculated that of the keratin cDNA duplexes with T_m lower than 85° at a C_0t of 6×10^1 mol s l⁻¹, more than 70% of these same molecules have T_m greater than 85° at a C_0t of $1\ 2\times10^4$ mol s l⁻¹

Interspersed unique and repetitive sequences in keratin genes

The striking features of the results are the greater apparent rates of formation of keratin cDNA duplexes when assayed with HAP compared with nuclease Sl and the progressive increase in average thermal stability of the duplexes with
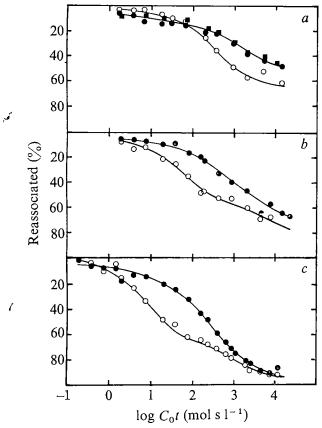
Fig 3 Reassociation of keratin cDNA with excess chick erythrocyte nuclear DNA Chick erythrocyte DNA, prepared as described[51], was sonicated (mean length 0 16 μm) Reaction mixtures (in NETS) contained 9 1 mg ml⁻¹ sonicated DNA plus about 35,000 c p m ml⁻¹ keratin cDNA, ¹²⁵I-chick erythrocyte-DNA (ref 39) (specific activity 1×10^5 c p m μg⁻¹), or ³²P-labelled chick embryo DNA, and were denatured (100 °C for 5 min) before incubation at 60 °C to the C_0t values shown For nuclease Sl assays in low salt buffer, 50 μl aliquots were diluted into assay buffer and incubated with or without nuclease Sl (150 units) for 30 min at 45 °C Nuclease Sl assays in high-salt were identical except that the assay buffer contained 0 3 M NaCl and incubation was at 37 °C HAP assays were as in Fig 1b After Sl digestion or HAP fractionation, all samples for any one curve were made up to the same final DNA concentration before TCA precipitation to avoid errors resulting from differential quenching Normalisation factors are shown as for Fig 1 a, Nuclease Sl assays in low-salt ●, ¹²⁵I-DNA (0%), ■, ³²P-DNA (0%), ○, keratin cDNA (0%) b, Nuclease Sl assays in high-salt ●, ¹²⁵I-DNA (14%), ○, keratin cDNA (0%) c, HAP assays ●, DNA (A_{260}) (12%), ○, keratin cDNA (10%)

increasing C_0t The data can be explained if there are multiple homologous keratin genes in the chick genome each containing two distinct sequences of approximately equal length, namely (1) a similar but non-identical sequence common to all the keratin genes ('reiterated sequence'), covalently attached to (2) a sequence so different in base sequence to that of any other keratin gene ('unique sequence') that it seems to be unique in the genome under the experimental criteria used here

The rapid transitions of keratin cDNA observed on HAP (compared with nuclease Sl in low salt conditions) can be explained as follows The reiterated sequences cross-hybridise early in the reaction (Figs 1b and 3c), forming duplexes which bind the entire molecule to HAP (ref 3) The unpaired unique regions would, however, be completely susceptible to nuclease Sl The low thermal stability of these duplexes indicates that about 20% of the base pairs are mismatched[42] The slow formation of duplexes of high thermal stability (Figs 2b and 4b) would result from hybridisation of the unique regions to their exact complements, forming concatenates of the early structures These duplexes would be stable to nuclease Sl

On this model, two distinct transitions would be expected after assaying hybridisation with nuclease Sl, the faster corresponding to the rapidly formed duplexes of the reiterated sequences and the slower corresponding to the unique sequences This was not observed when nuclease Sl assays were carried out in low salt (Figs 1a and 3a) The single transition observed, however, occurred over three decades of R_0t (Figs 1a and 3a), a sure indication of heterogeneity[3] Under high salt conditions in the Sl assays two distinct transitions were observed with keratin cDNA–DNA duplexes (Fig 3b) The extent of this early transition (Fig 3b) suggests that the reiterated sequences occupy about 50% (that is, about 250–300 bases) of the cDNA molecules

The difference in Sl-resistance of early keratin cDNA–DNA duplexes in low and high salt (compare Fig 3a and b) can be explained if the mismatched duplexes are degraded to an extent of about 50% in low salt and are completely resistant in high salt Therefore, although two distinct transitions are not apparent in Figs 1a and 3a, the first 20–30% of the observed transition can be attributed to mismatched duplexes of the reiterated region which are partially degraded by nuclease Sl, resulting in a final extent of only 70–80% resistance of the hybrids to nuclease Sl (Fig 1a) The presence of high-salt concentrations did not protect cDNA–mRNA hybrids against degradation by nuclease Sl (data not shown) The smaller slow transitions of keratin cDNA observed on HAP (Figs 1b and 3c) could result from thermal scission, incomplete transcription, impurities in mRNA, or a combination of these factors

Estimation of the number of molecular species in embryonic chick keratin mRNA

The kinetic complexity of the unique sequences in keratin mRNA estimated by the nuclease Sl procedure (Fig 1a) was about 25 times that of globin mRNA, and estimated by the rate of formation of hybrids eluting from HAP at greater than 80° (Fig 2b) was about 18 times that of globin mRNA Since the α and β chains of rabbit globin mRNA and cDNA do not cross hybridise[30-32] the complexity of globin mRNA is about 1,100 bases, giving an apparent complexity of about 20,000–28,000 bases for keratin mRNA Since these unique sequences only occupy about half of the mRNA, this adjusts to 10,000–14,000 bases when corrected for the actual concentration of unique sequences instead of total mRNA, equivalent to 25–35 different unique sequences about 400 bases long This estimate of 25–35 different mRNA species is compatible with the minimal number of 19 to 25 keratin chains[21,22] detected in embryonic chick feather keratin

Estimation of the number of keratin genes in the chick genome

Keratin cDNA reannealed to chick DNA about two to three
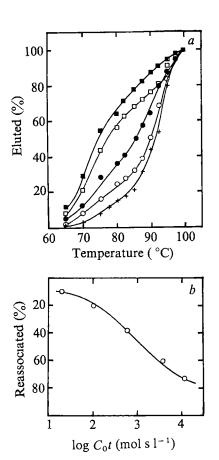
Fig 4 Thermal stabilities of keratin cDNA–DNA duplexes Reaction mixtures as in Fig 3 were treated as in Fig 2, except that carrier DNA was not added until after fractionation on 2 0 ml HAP columns *a*, Keratin cDNA–DNA duplexes after incubation to C_0t values (mol s l^{-1}) of ■, $1\,9\times10^1$, □, $1\,0\times10^2$, ●, $5\,7\times10^2$, ○, $3\,06\times10^3$, +, $1\,15\times10^4$ *b*, Rate of formation of keratin cDNA–DNA duplexes eluting from HAP at greater than 85 °C, calculated from the data of Figs 3*c* and 4*a* as described for Fig 2*b*

Nature of the unique and reiterated sequences in keratin genes

The limited available sequence data[21] indicate that individual embryonic chick feather keratin chains dffer from each other in amino acid sequence by a maximum of about 8% and that most of these differences are the result of single base changes In the conditions used here, stable hybrids form between mouse globin cDNA and rabbit globin mRNA (ref 43), although mouse and rabbit globin chains differ by 19% in amino acid sequence Keratin structural genes should therefore cross hybridise with each other unless mutations which do not change the amino acid specified (relative to those that do change the amino acid specified) occur in keratin structural genes much more frequently than in globin structural genes It is therefore likely that the reiterated sequences are the keratin structural genes, that there are about 100–240 of these in the haploid chick genome, that on average they have diverged from each other by about 20% in base sequence, and that many of them are not expressed, or expressed at a very low level in the embryonic feather

If this interpretation is correct, it follows that the unique sequences are the untranslated regions and that these have diverged at a much greater rate, to the extent that each seems to be unique in the haploid genome A similar situation has been observed in the case of sea-urchin histone genes[44] In this case the spacer sequences are also thought to have diverged at a greater rate than the histone structural genes[44] This also seems to be true for the spacer sequences of ribosomal genes[45] Preliminary results indicate that shorter keratin cDNA molecules obtained using *Escherichia coli* DNA polymerase I to copy keratin mRNA contain only the unique sequences, suggesting that the unique sequences are localised toward the 3' end of keratin mRNA

Recent studies indicate that most eukaryotic mRNAs are transcribed from unique sequences[12-14,30,37,38,46,47], although a small fraction which seems to be transcribed from repetitive sequences has also been reported[30][46-48] In contrast to the situation in keratin mRNA, however, these repetitive and single-copy transcripts are not covalently interspersed[46,47] Whereas the genes for duck globin seem to be adjacent to repetitive sequences in the genome[50], keratin genes seem to be the first example of repetitive sequences of known function which are interspersed with unique sequences The occurrence of both unique and repetitive sequences in keratin genes should greatly facilitate studies on their arrangement in the chick genome

times faster than the unique sequence fraction of chick DNA as determined by the nuclease S1 procedure in low salt, indicating that the unique regions are reiterated two or three times The strong dependence of the kinetics on the salt concentration in the nuclease S1 assays suggests that this could be an overestimate This interpretation is supported by the kinetics of formation of keratin cDNA–DNA duplexes eluting from HAP at greater than 85° (Fig 4*b*), which indicate that the unique sequences occur only about once in the haploid genome

The reiterated sequences in keratin mRNA hybridised to keratin cDNA with a $R_0t_{\frac{1}{2}}$ of 9×10^{-4} mol s l^{-1} when measured on HAP (Fig 1*b*), about four times slower than the rate expected for a single perfectly complementary species Mismatching of about 20% (ref 42) would lower the reaction rate by a factor of about four[42], giving a corrected apparent complexity of about one for the reiterated sequence This implies that most if not all keratin mRNA species share the sequence

The reiterated sequences reannealed to chick DNA about 60 times as fast as the unique sequence fraction of chick DNA when determined on HAP (Fig 3*c*) and about 25 times as fast when assayed with nuclease S1 in high salt (Fig 3*b*) When corrected by the factor of four for the reduction in rate caused by mismatching, the reiteration frequency of this sequence seems to be 100–240 If, however, the low T_m of duplexes of these regions is a consequence of the sequences being very short rather than poorly matched, the factor of four may not apply and the estimate may be too high This would not invalidate the conclusion that there are two distinct sequence classes present in each keratin gene

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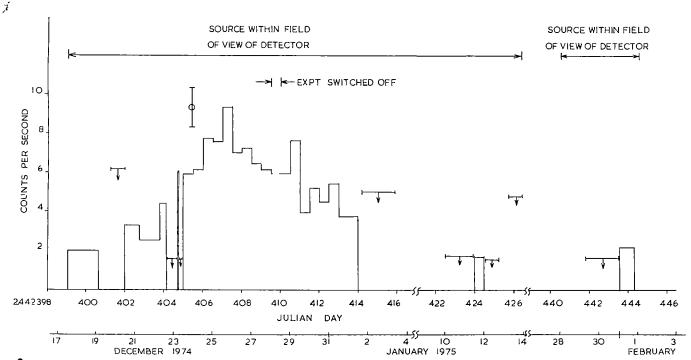
letters to nature

Variable X-ray source near Cen X-3

THE satellite Ariel V, launched on October 15, 1974, as part of the US-UK collaborative space programme, contains a rotation modulation collimator experiment designed to measure the positions of X-ray sources, which was built by the Mullard Space Science Laboratory The experiment responds to X rays in the quantum energy range 3 1–9 3 keV, the area of the proportional counters being 256 cm² which, allowing for grid transmission, counter efficiency and so on, leads to an effective area of 102 cm² at 6 0 keV The pitch-to-separation ratio of the collimator grids is 112, corresponding to a full width at half maximum (FWHM) of the image of a point source of 15′ (ref 1) The spin axis was manoeuvred to point towards a number of sources in succession in the Centaurus region, including the important binary source Cen X-3, from December 17, 1974 to January 31, 1975, to carry out position and spectrum determinations Soon after arriving at the first pointing direction in the sequence, a correlation map produced by overlaying data from orbits obtained on December 17–19 showed the presence of a new source not listed in the Uhuru (3U) catalogue, which we have designated A1118-61 from the position given below The field of view of the experiment is 17°

Fig 1 Counting rate of variable source in Centaurus region as a function of time after subtraction of background The symbol circle with a vertical bar indicates typical ±1σ values near the maximum intensity, as noted in the text this arises partly from fluctuations in source strength on successive orbits The standard deviation of the fluctuations in background level is one-half of this or less The variation in the magnitude of the upper limits is because of changes in the distance of the source from the spin axis, except on December 20 for which the data were of relatively poor quality

FWHM, so that the light curve could be followed over an interval of 46 d The light curve is given in Fig 1 in units of counting rate in our detector, which can be converted approximately to Uhuru counts by multiplication by 8 In the initial and final periods of observation, the source was rather weak, and could be detected at a satisfactory significance only by overlaying orbits, with a consequent reduction in time resolution The latter improved to one orbit (101 min) when the source was near its maximum brightness We have, for clarity, averaged the intensity over 12 h in this period, but significant intensity variations, exceeding those attributable to counting statistics, were observed in the one-orbit averages Where an upper limit only is given, it corresponds to three standard deviations of the background level

Both at the beginning and at the end of our observation period the emission from the source was detectable It is possible that the event we observed is a rather strong flare of the kind observed in Sco X-1 On the other hand, if it is assumed that the emission rose to the level we observed immediately before our observations, and that it decayed further afterwards, then the X-ray light curve strongly resembles that shown at optical wavelengths by some moderately fast novae, which may exhibit a 'pre-maximum halt', a dip and a rise to the maximum The structure in the light curve on JD 2, 442, 404–5 is real, the emission before and after the sharp peak being at a low level, on which we could place only upper limits The intensity change on either side of the dip exceeds 5 σ, and would correspond to the dip in the nova light curve The X-ray light curve from this source differs considerably from that of Cen X-4 (ref 2) which showed no pre-maximum dip At times when the pointing direction was sufficiently close, another experiment on Ariel V obtained data on the short-period fluctuations which are described in the accompanying letter[3]

We have obtained a position for this source Our experiment is most accurate when another X-ray source of accurately known position is simultaneously in the field of view and we hoped to use Cen X-3 for this purpose Unfortunately from December 17 to January 10, Cen X-3 was in an 'extended low' After this its intensity increased only rather slowly and by this time the brightness of the new source had diminished considerably We were, however, able to obtain four simultaneous observations of the two sources We also observed two other Uhuru sources, 3U1223−62 and 3U1254−69, and a second, new, variable source By considering all the simultaneous observations of these sources with one another and with Cen X-3, we have been able to improve this estimate of the position, with the final result RA 11 h 18 min 59 ± 13 s, declination −61° 35 3 ± 1 8′ These are 1950 coordinates, the errors being ±2σ The position used for Cen X-3 was RA 11h 19 05 min, declination −60° 21′ (ref 4) We note that our observations place both the 3U sources outside of and to the north-east of their 3U error boxes, though by varying amounts, which may indicate a systematic error in our results which is not allowed for in the position error we have quoted We have looked for such a systematic error and failed to find any direct evidence for it On the basis of the position of 3U1254–69, which has the smallest error box, we estimate the maximum value of any error at 4′, such that the source would lie to the south-west of the position given

Although the X-ray emission may now have decayed below the value we first observed, we suggest that optical observation of possible candidate stars may still be worthwhile in view of the periodicity described in ref 3

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Observations of a transient X-ray source with regular periodicity of 6.75 min

During planned observations, by the satellite Ariel V, of the X-ray source Cen X-3 (3U1118-60), a previously unreported source nearby was detected by the collimated proportional counter of 100 cm^2 provided by the Mullard Space Science Laboratory The source was monitored over a period of 39 d between December 19, 1974, and January 27, 1975 Here we report measurements of the light curve, spectrum and of a regular variability with period of 6 755 ±0 010 min The position of the source is given in the accompanying letter[1]

The intensity of the source is shown plotted against time in Fig 1 for the period December 19, 1974 to January 1, 1975, during which time Cen X-3 was in an extended low state The first observations on December 19–20, 1974, indicated a weak source of 0 006 photon cm^{-2} s^{-1} keV^{-1} in the energy range 3–30 keV The intensity increased by a factor of four during the period December 20–25, 1974, peaking at a strength of 0 02 photon cm^{-2} s^{-1} keV^{-1} Subsequently, the intensity decayed with an e-folding time of approximately 7 d The intensity at the time of the final observation on January 27, 1975, had fallen to 0 004 photon cm^{-2} s^{-1} keV^{-1} This and other data after January 1, 1975, were taken during Cen X-3 binary off periods

The last two points on Fig 1a show an increase in intensity and are coincident with a predicted[7] binary transition of Cen X-3 from off to on Immediately after this, the spacecraft moved away from Cen X-3 for 7 d It seems likely that this marks the first turn-on of Cen X-3 after the extended low

Spectra of the source have been obtained with 32 pulse-amplitude channels analysing the output from the proportional counter in two energy ranges, 1 5–15 keV and 3 0–30 keV The data discussed here refer predominantly to the former

Table 1 Best-fit parameters

Phase	Coefficient	Power	N_H	χ^2
Before	0 11±0 02	1 10±0 07	(5 5±0 8)×10^{22}	3 2
During increase	0 21±0 03	1 07±0 07	(6 6±0 8)×10^{22}	3 2
At maximum	0 22±0 03	0 91±0 05	(5 8±0 6)×10^{22}	5 3
During decay	0 22±0 03	1 14±0 05	(5 9±0 6)×10^{22}	4 7

Spectra have been obtained from the different regions of the light curve—before and during the increase, at maximum and during the decay In each case, the data are best fitted with a power law spectrum rather than an exponential or blackbody spectrum No such simple model spectrum satisfactorily describes the data, however The best-fit parameters are shown in Table 1, the errors being (χ^2+1) values

Except for a marginally significant flattening of the spectrum at peak intensity, the spectral shape above 4 keV is constant
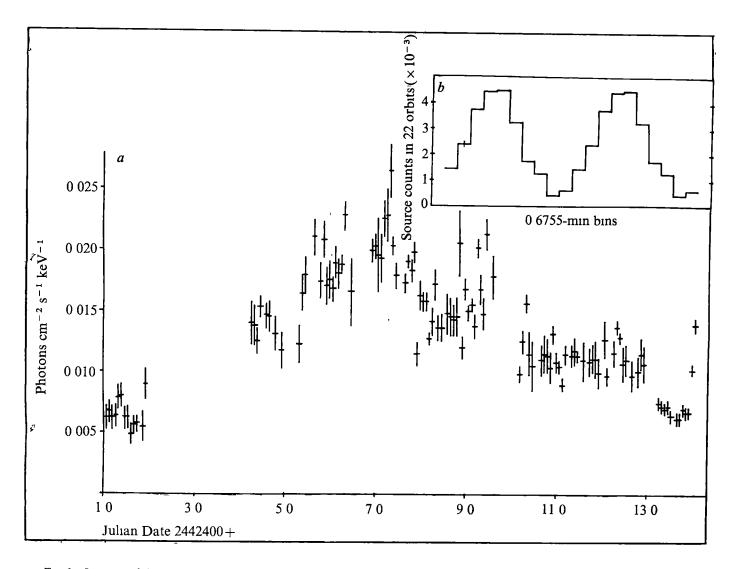
Fig 1a Intensity of the source averaged over one orbit (1 6 hours), as a function of time (1 day ≈ 14 orbits) Errors shown are statistical There may be an additional uncertainty of up to 6 % caused by errors in the spacecraft attitude solutions *b* 6 755-minute period light curve of the source The error shown is statistical

throughout the observations The low energy spectrum, below 4 keV, shows a photon deficiency, independent of source model, indicating considerable absorption—amounting to 6×10^{22} N_H atoms cm^{-2}, assuming the abundances of Brown and Gould[2] This value did not change during the observation Measurements of 21-cm emission[3] give a column density to the edge of the Galaxy, in the direction of this source, of $1\,2 \times 10^{22}$ atoms We conclude, therefore, that there is significant absorption from material local to the source

The source intensity was measured on December 31, 1974, with a time resolution of 64 s Periodic variability was apparent in the raw data and by folding the data from successive orbits with trial periods near that observed in the raw data, a period of $6\,75 \pm 0\,03$ min was obtained Data were also obtained with a time resolution of 48 s on January 21 and 23, 1975, when the source strength had decayed to 0 005 photon cm^{-2} s^{-1} keV^{-1} The regular variability was again present with unchanged period, within the limits of the uncertainty of $\pm 0\,03$ min In each case, the depth of modulation was large—greater than 80%

Combining all these data allows the period to be refined to $6\,755 \pm 0\,010$ min The light curve of this periodicity is shown in Fig 1*b* The depth of modulation is 85 ± 3%

As the 6 755-min periodicity and large depth of modulation persisted, unchanged, within the accuracy of our measurement,

to a stage at which the source intensity had fallen below the level of the initial sighting, it is likely that this periodicity is a constant characteristic of the source and not a transient periodicity associated with the flare

Another significant feature of this observation is the constancy of the spectral shape, both in the soft X-ray absorption and higher energy slope

Regular variability in X-ray sources may be divided into two classes—those with periods of a few seconds or less, believed to be associated with the rotation period of a compact star, such as a neutron star, and those with periods of a few hours or more, associated with the orbital motion of a binary system The present periodicity falls between these two groups

The quasi-sinusoidal nature of the light curve seems to rule out an origin for the pulses from beamed radiation from rotating neutron stars, and square wave modulation, as might occur in an eclipsing binary system may also be excluded

The light curve of the present periodicity is not unlike that of the 4 8-h periodicity of Cyg X-3[4] In a model for Cyg X-3[5,6] the X-ray source is proposed to be a white dwarf associated, in a binary system with a red dwarf The X-ray modulation is the result of electron scattering in a column of material whose optical depth varies with the orbital phase of the white dwarf about its companion
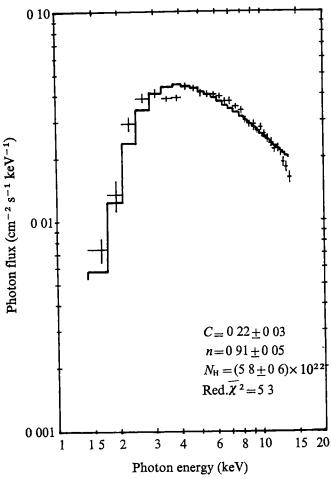
Fig 2 Comparison between the sum of the data collected during five consecutive orbits over the period of maximum intensity and best fitting power law spectrum $(I = C \exp(-N_H)E^{-n})$

If the 6 755-min period is attributable to orbital motion of a binary system, the system must be appreciably more compact than the Cyg X-3 system In this new source, Ariel 1118-61, both objects must be compact

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Occurrence of diamond in a mica–garnet lherzolite xenolith from kimberlite

ALTHOUGH graphite has previously been reported from upper-mantle garnet lherzolite xenoliths from kimberlite intrusions, diamond has not previously been found in upper-mantle garnet lherzolite xenoliths from kimberlite intrusions Diamond of primary terrestrial origin is found mainly in the rare ultramafic, potassic rock, kimberlite Although the precise source of most diamonds in kimberlite is unknown it is generally assumed that most of the diamonds originate from the kimberlite matrix itself A few have, however, been found enclosed in eclogite xenoliths of widely varying composition[1-3] and also in rare xenoliths of garnet serpentinite from the Aykhal kimberlite diatreme in Siberia[4] We report here on the occurrence of diamond in a xenolith of garnet lherzolite, the material generally believed to dominate the Earth's upper mantle on density, seismic and experimental grounds

The xenolith (BD2125) was collected from the kimberlite of the Mothae kimberlite diatreme[5] in northern Lesotho During preparation of a thin section, a small, extremely hard grain was encountered Preliminary tests (Moh's hardness > 9, insoluble in hydrofluoric acid HF) and subsequent single-crystal X-ray diffraction proved that it was diamond The crystal is irregular with maximum and minimum widths of 0 8 and 1 4 mm, respectively, it weighs 2 7 mg The precession X-ray patterns show twinning on (111), but the position of the twin boundary is unknown The surface has one near-planar face—identified as (111) by X rays—but is mostly irregular, ranging from rounded parts to irregular hackly parts, perhaps showing multiple exposures of cleavage surfaces or growth features A prominent re-entrant at one end may result from the twinning The diamond is essentially colourless Surface and internal impurities occur, the latter including thin veils and two thin, flat rods about 0 01 mm long lying parallel to the flat face One of the rods is yellow-brown and the other is colourless Two more rods lie close to the surface, and at least one (probably several) body projects inwards from the surface

The host xenolith is angular 6 × 8 × 9 cm The rock is very fresh and has a distinct fabric, with the long axes of the olivine and orthopyroxene grains showing preferred orientation parallel to the longest axis of the xenolith In the terminology of Bouiller and Nicolas[6] the texture is coarse tabular There are some triple boundaries of approximately 120° between adjacent grains, which often have curved grain boundaries, some orthopyroxenes and olivines show undulose extinction This complex texture indicates recrystallisation towards an equilibrium texture (though this is not quite achieved), perhaps with late-stage deformation The mode of the rock is variable, with olivine and orthopyroxene always dominant, garnet is a consistent minor mineral, but clinopyroxene is relatively rare and sporadic The olivine and orthopyroxene grains measure up to 6 mm long, and the garnets are up to 4 mm across, the clinopyroxene grains do not exceed 3 mm in length The garnets are surrounded by a narrow (0 2 mm) reaction rim of clinopyroxene, orthopyroxene and equant brown spinel Three mica crystals were found in the four polished thin sections prepared for electron microprobe study Two of them abut each other next to the reaction rim of a garnet, are about 1 × 0 5 mm in size, show light-brown pleochroism, and are multiply kinked A third crystal in another thin section is similar in all respects

Electron microprobe analyses showed uniform compositions irrespective of texture Representative analyses are given in Table 1 The olivine (Fa_7) and enstatite (Fs_6) are highly magnesian, the latter containing small amounts of Al_2O_3, Cr_2O_3 and CaO The garnet is pyrope-rich with 5 5 weight% Cr_2O_3 and little TiO_2, it thus resembles the pyrope found in most common garnet lherzolites It differs, however, from the garnets in the diamond-bearing garnet serpentinites from the Aykhal kimberlite, in which the two analysed pyropes contain 8 2 and 14 1 weight% Cr_2O_3, respectively In addition, one of the Aykhal garnets contains only 1 9 weight% CaO which, although in this respect resembles some garnet inclusions in diamond[3,7], serves to set it apart from the garnet in the xenolith discussed here The clinopyroxene is a sodic diopside containing small amounts of Al_2O_3 (1 9 weight%), Na_2O (1 5 weight%), Cr_2O_3 (1 6 weight%) The mica is a phlogopite with low TiO_2 (0 14 weight%) and intermediate Cr_2O_3 (0 86 weight%) These values fall in the range for primary micas from peridotite xenoliths[8] In short, the mineral chemistry is quite consistent with that of other upper-mantle, granular, mica-garnet lherzolites[9]

Table 1 Silicate analyses of the xenolith (BD 2125)					
	1*	2	3	4	5
SiO$_2$	41 5	40 9	57 9	55 0	41 7
TiO$_2$	0 07	0 00	0 01	0 02	0 14
Al$_2$O$_3$	19 9	0 03	0 79	1 88	12 9
Cr$_2$O$_3$	5 50	0 03	0 33	1 65	0 86
FeO	5 95	7 00	4 19	1 80	2 45
MnO	0 32	0 08	0 11	0 07	0 01
MgO	20 5	50 7	36 2	17 8	26 8
NiO	0 01	0 38	0 10	0 02	0 25
CaO	5 66	0 03	0 53	20 4	0 00
Na$_2$O	0 02	0 01	0 10	1 55	0 06
K$_2$O	0 00	0 00	0 00	0 03	10 3
Total	99 4	99 2	100 2	100 2	(95 5)
Si	5 971	5 992	7 900	7 906	5 868
Al^{IV}		0 006	0 100	0 094	2 132
Al^{VI}	3 377		0 027	0 226	0 010
Ti	0 007	0 000	0 001	0 002	0 015
Cr	0 626	0 003	0 035	0 188	0 096
Fe	0 716	0 840	0 477	0 216	0 288
Mn	0 040	0 010	0 013	0 009	0 001
Mg	4 406	11 08	7 353	3 829	6 999
Ni	0 001	0 045	0 011	0 002	0 028
Ca	0 873	0 005	0 077	3 145	0 001
Na	0 004	0 003	0 026	0 433	0 016
K	0 001	0 000	0 000	0 005	1 852
O	24	24	24	24	(22)

* 1, garnet, 2, olivine, 3, orthopyroxene, 4, clinopyroxene, 5, mica Electron microprobe analyses by Chicago 1974 11-element system Special analyses for minor elements gave TiO$_2$ garnet, 0 075 weight%, olivine, 0 002 weight%, orthopyroxene, 0 014 weight%, clinopyroxene, 0 021 weight% Cr$_2$O$_3$ olivine, 0 029 weight% Na$_2$O garnet, 0 021 weight%

The Ca/(Ca+Mg) ratio of diopside and the Al$_2$O$_3$ content of enstatite provide an estimate[10] of temperature and pressure for pyrope-lherzolites, but the details are controversial The Ca/(Ca+Mg) ratio of 0 451 ± 0 002 for the clinopyroxene from this xenolith indicates an equilibration temperature of 1,040 °C (using the Di–En join of Davis and Boyd[11]) and 980 °C (using new electron microprobe data[12] for the Di–En join at 3 × 10^5 kPa) Four analyses using a Di$_{85}$Jd$_{15}$ standard gave Ca/(Ca+Mg) ratio of 0 449, 0 451, 0 452, 0 452) The empirical fit of Wood and Banno (ref 13, equation 27) to cover the substitution of the minor elements, especially Fe, Al, Cr and Ba, leads to a positive correction of about 70 °C Preferring the direct chemical data[12], however, we tentatively propose an equilibration temperature of 1,050 °C The Al$_2$O$_3$ content, 0 79 weight%, is too low to read from Fig 2 of MacGregor[14], and the spacing of the isopleths is nonlinear An empirical extrapolation of experimental data by Boyd and England together with the correction for minor elements (ref 13, equation 17) yields an estimated pressure of 5 2 × 10^5 kPa, substitution of MacGregor's[14] data yields a value of 4 6 × 10^5 kPa (B J Wood, personal communication) The shield geotherm of Clark and Ringwood[15] passes through 150 km at 1,050 °C, which is fairly consistent with the estimated pressures, and is just inside the stability field of diamond

Apart from the intrinsic interest of this first published record of diamond in a mica–garnet lherzolite, several points should be discussed

First, the irregular rounded shape of the diamond (apart from the flat face which may be a cleavage artefact from preparation of the thin section) is perhaps relevant to the observation[16] that some diamonds are a combination of two distinctive growth forms One represents an earlier stage in which the habit is rounded or mamillary (except for limited octahedral flat faces), and the other is an overgrowth with a distinctly octahedral habit With the proven existence of a rounded diamond in garnet lherzolite, it may be possible to speculate that during the disruption of garnet lherzolite into a kimberlite melt, small rounded diamonds act as nuclei for diamond which precipitates from the magma with a regular habit

Second, the xenolith we have discussed contains phlogopite and the inferred pressure and temperature fall at the upper end of the range for mica-bearing lherzolites[10] Two of the lherzolites described by Boyd[10] contain graphite as well as phlogopite, in contrast to his phlogopite-free specimens which contain no graphite and have inferred pressures and temperatures that place their equilibration within the stability field of diamond If the phlogopite in our xenolith grew in equilibrium with the diamond, it may be inferred that the phlogopite field does extend into the stability field of diamond

Finally, the presence of graphite in garnet lherzolite xenoliths could arise from either primary growth on the low-pressure side of the graphite–diamond curve or from the breakdown of diamond attendant on upward movement of the host lherzolite out of the diamond stability field or from a combination of both factors The latter would occur in a manner analogous to the breakdown of pyrope in lherzolites during their rise to the Earth's surface[17] This may be brought about by upwelling of an upper-mantle diapir connected with kimberlite magmatism (see ref 18)

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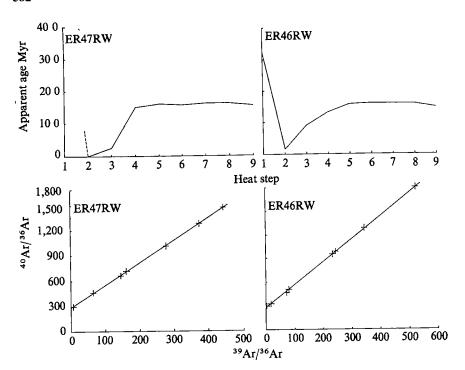
Age of a new carbonatite locality in northern Kenya

THE ages of carbonatites which occur within the Suregei–Asille Volcanics of northern Kenya cannot be obtained at present by direct radioisotope dating, but can be derived from the ^{40}Ar/^{39}Ar K–Ar isochron ages of intercalated felsic ignimbrites The Suregei–Asille Volcanics crop out over an area of some 1,000 km^2 to the north-east of Lake Rudolf They rest unconformably on the Precambrian basement and comprise several volcanic formations The rock types include flood basalts, Strombolian cinder cones, aa- and pahaoehoe-flows varying in composition from ankaramitic basalt to trachyte, felsic airfall and ash-flow deposits, lahars, carbonatite volcanics, and a variety of reworked volcanic ashes, volcaniclastic and fluviatile sediments

Fig. 1 $^{40}Ar/^{39}Ar$ age spectra and K–Ar isochrons obtained from samples of juvenile volcanic sanidine separated from two strati-graphically adjacent alkaline felsic ignimbrites (ER47RW and ER46RW) from the Gum Dura Formation of the Suregei–Asille Volcanics, northern Kenya In each case the analytical data are plotted both as an age spectrum and as an isochron[2,10] The shape of the age plateau obtained from ER46RW suggests the presence of a minute discrepancy caused by argon loss, but this is seen to have had a minimal effect on the isochron determination The age plateau and K–Ar isochron from ER47RW are perfect examples of the results to be expected from completely non-discrepant dating samples

An important marker horizon within the Suregei–Asille Volcanics is a thin welded ignimbrite of considerable extent This distinctive felsic ignimbrite forms part of the Gum Dura Formation (R T W, unpublished) Within this formation, fine-grained carbonate rocks of volcanic origin are particularly important These newly discovered carbonatites are of exceptional petrogenetic interest first they seem to be pyroclastic rocks emplaced by ash-flow and air-fall eruptions, second, their association is with alkali-basalts and felsic ignimbrites rather than with feldspathoidal lavas and undersaturated alkaline intrusive rocks Elsewhere in the Suregei–Asille Volcanics there are discrete volcanic centres, lava flows and pyroclastic rocks of similar carbonate mineralogy[1] Recrystallisation and silicification are commonly developed in these carbonatites The presence of thick, carbonate-rich horizons within the volcanics which surround and underlie the East Rudolf Plio–Pleistocene sedimentary basin may explain, at least in part, the frequent occurrence of calcite mineralised fault planes, calcite veining and the widespread presence of calcite cementation in the rocks of the basin

Two ignimbrites within carbonatites of the Gum Dura Formation were sampled for dating using the $^{40}Ar/^{39}Ar$ single sample K–Ar isochron technique[2] The first sample (ER47RW) was taken from the prominent marker horizon already mentioned The second (ER46RW) is a check sample from a less extensive ash-flow unit which underlies the first at the collection locality (ref BH810850 on the Kenya National Grid), a river gorge on the Il Dura branch of the upper Il Eriet, some 5 km south of the Kenya–Ethiopia border Discoloration in sample ER46RW suggests that it has been subjected to some kind of weak hydrous alteration that did not penetrate the overlying, more strongly welded horizon Concentrates of euhedral juvenile volcanic sanidine for dating were extracted from each rock sample by crushing and hand picking using a stereoscopic polarising microscope

The results of full $^{40}Ar/^{39}Ar$ step-heating age analysis of the two sanidine concentrates are illustrated in Fig 1 The results from the first dating sample (ER47RW) indicate that it contains only one age component, producing a single plateau on the age spectrum and defining a perfect K–Ar isochron of apparent age $16\ 22\pm0\ 10$ Myr ($^{40}Ar/^{36}Ar$ intercept value 288 ± 4) The

shape of the age spectrum obtained from the check sample (ER46RW) suggests that a slight discrepancy resulting from argon loss may be present, although the isochron plot reveals that any argon loss is virtually insignificant and a nearly perfect K–Ar isochron of apparent age $16\ 14\pm0\ 13$ Myr ($^{40}Ar/^{36}Ar$ intercept value 280 ± 6) was also obtained from this sample The two K–Ar isochron ages are in agreement within the experimental limits, therefore, the marginally superior age of $16\ 22\pm0\ 10$ Myr obtained for the prominent marker horizon can be accepted with confidence Both isochrons intersect the $^{40}Ar/^{36}Ar$ axis at a ratio of less than $295\ 5$, indicating the presence of initial argon which had an argon isotope ratio unlike that in the modern atmosphere thus any conventional K–Ar age determinations or calculations relating to these samples will produce discrepantly low apparent age values The K–Ar isochron age accepted for the upper felsic ignimbrite in the Gum Dura Formation is consistent with, but considerably superior to, previous K–Ar dates from the Suregei–Asille Volcanics In particular, it is in agreement with both the average, conventional K–Ar apparent age of $17\ 3\pm1\ 4$ Myr obtained from a basalt slightly lower in the succession north of the Buluk Gap[3], and with the average, conventional K–Ar apparent ages of $14\ 1\pm1\ 4$ Myr, $12\ 3\pm1\ 4$ Myr and $11\ 6\pm0\ 5$ Myr obtained from basalts higher in the succession along the Suregei Cuesta[4,5]

This precise, geologically acceptable K–Ar isochron age, confirmed from an adjacent but independent ash-flow, is an excellent example of the efficacy of the $^{40}Ar/^{39}Ar$ single sample (or step-heating) K–Ar isochron dating technique In addition, the generation of reproducible, perfect and near perfect isochrons from volcanic sanidines in this study makes it unlikely that complex age spectra obtained from similar analyses of the same mineral (for example, sanidine concentrates separated from pumice occurrences in the reworked tuffs of the East Rudolf sedimentary basin[4,5]) can be explained away or rejected as artefacts of the $^{40}Ar/^{39}Ar$ irradiation technique

The association between voluminous felsic volcanism and carbonatitic ash-flow volcanism in the Gum Dura Formation is unique The firm dating of this extremely unusual episode of carbonatite magmatism at $16\ 22\pm0\ 10$ Myr places it in the early Middle Miocene At this time the Suregei–Asille region

formed part of a shallow structural depression lying between the rising Ethiopian and Kenyan basement swells[6] Volcanism has been important on both swells as well as in the intervening Turkana depression since their inception If, as seems likely, these swells and the rift valleys that eventually developed across their crests can be regarded as the surface expressions of two adjacent mantle plumes, then a detailed knowledge of the sequence and changing petrography of the magmas erupted throughout this East African Rift Province is an essential prerequisite to our understanding of these phenomena Volcanism on the Kenya swell began with the appearance during the Lower Miocene of a number of isolated melanephelinite-phonolite central volcanoes Further north, in the Turkana depression and in Ethiopia, extensive alkali-basalt lineage eruptions with associated ignimbrite volcanism became prevalent around this time

Dissection of some of the large, Miocene central volcanoes in western Kenya and eastern Uganda has revealed that they possess intrusive ijolite–carbonatite cores (for example, Napak[7,8]) The dating of these intrusive carbonatites and their associated ijolites has proved to be particularly difficult It has been suggested from purely geological considerations that an age within the latter part of the range 22–16 Myr may be more reasonable for the central complex of Napak than are those obtained from direct conventional K–Ar age analysis of the core rocks[9] If it can be shown that carbonatite magmas of very different petrogenesis and volcanic association were available contemporaneously in East Africa during the Middle Miocene, then it may be possible to explain these differences in terms of structural setting in relation to the mantle plumes that have dominated East African geology over the past 25 Myr

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Origin of magnesium and potassium ions in Lake Vanda, Antarctica

THE Dry Valley area of southern Victoria Land is one of the greatest ice-free areas in Antarctica and contains lakes and soils which are noted for their saline character Particular attention has been focused on the origin of these salts, first because it contributes to an understanding of Antarctic weathering and soil formation, second because it is related closely to the glacial history of the Dry Valley area, and third because it is connected with marine invasion and the Pecten gravels[1] in Wright Valley

Suggested sources for the salts in the Dry Valley area include marine invasion of the valleys, atmospheric precipitation and rock weathering Most published chemical studies reject marine invasion of Wright Valley because of the hydrological barrier created by the valley threshold, although Webb[2] presented convincing palaeoecological evidence for marine penetration of the valley Nakai[3] concluded from the stable sulphur isotopic measurements of sulphates around Lake Vanda, Wright Valley that sulphates in the lake sediments of Hole No 4 of the Dry Valley Drilling Project[4] and sulphate minerals around the lake originated from seawater, indicating marine invasion or atmospheric precipitation The origin of salts in Lake Vanda has also been discussed on the basis of the distribution of evaporites and soil salts[5]

Many workers have analysed six kinds of ions—Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-}—in the saline lakes of the Dry Valley area These ions are also main components of seawater The ionic composition of the water in the lakes is, however, different from that of seawater There are extensive deposits of thenardite, halite, sodium nitre, calcite and gypsum around Lake Vanda Therefore, contemporary distributions of Na^+, Ca^{2+}, Cl^- and SO_4^{2-} are likely to bear little relation to primordial levels in the lake On the other hand, magnesium and potassium salts are not found around Lake Vanda except in rare occurrences of epsomite and dolomite Thus, contemporary distributions of Mg^{2+} and K^+ are likely to bear some relation to primordial distribution

Further evidence justifying selection of Mg^{2+} and K^+ for study arises from the types of salts formed during the concentration of brine The ground temperature in Wright Valley averages between $-25\,°C$ and $-30\,°C$ from March until September[6] Therefore, it would be more reasonable to suppose that salts deposit as a result of fractional freezing rather than by normal evaporation In frigid conditions, the concentration curves[7] for Mg^{2+} and K^+ in seawater undergoing progressive cooling exhibit a fairly constant relationship between 0 and $-45\,°C$ Values for the Mg/K ratio range from 3 to 4 There is a steady increase in concentration of both ions down to $-36\,°C$, further lowering of temperature causes sedimentation of magnesium chloride and potassium chloride, and a reduction in concentration These data were obtained during progressive freezing of brine samples, and the thermal history of the lake and its surroundings are probably different, in the natural situation it is probable that Mg^{2+} and K^+ would remain in residual brine without the crystallisation of magnesium and potassium salts Furthermore, if the ionic composition of primordial brine were similar to that of seawater, the Mg/K ratios would remain constant below $-36\,°C$ at which temperature the magnesium and potassium salts begin to crystallise Therefore, it is likely that, under prevailing temperature conditions, the Mg/K ratio was relatively undisturbed during the freezing of brine in the lake

Because of their high solubility any superficial deposits of magnesium and potassium salts on the surrounding land may be washed into the lake From the considerations mentioned already we suggest that the Mg/K ratio of this component will also remain close to the primordial value

Using the chemical composition data for samples of lake water collected from various parts of Lake Vanda[8] we classified the samples according to the values of the Mg/K ratio Figure 1 shows the plot of the value of the Mg/K ratio against the concentration of total salts (sum of six components) in lake water There are two types of brine in the lake water according to the values of the Mg/K ratio The boundary value is nearly 4, which is the same as that in seawater Since the value of the Mg/K ratio is nearly 1 in freshwater ponds in the Dry Valley area, Mg^{2+} and K^+ in samples for which the ratio is less than 4 probably originate from the weathering of rock and from sea
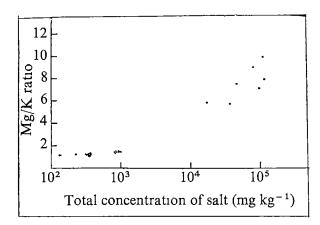
Fig 1 The Mg/K ratio against the concentration of total salts in water of Lake Vanda

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spray These samples exist mainly in the shallower parts of the lake, and are less dense than seawater Rock weathering and sea spray are possible sources for salts throughout the Dry Valley area and these processes are occurring at present

On the other hand, we suggest that Mg^{2+} and K^+ in samples for which the ratio is greater than 4 originate from seawater These samples mainly originate from parts of the lake deeper than about 45 m In comparison with a seawater distribution, however, Mg^{2+} is enriched relative to K^+

Although Mg^{2+} and K^+ have similar concentration characteristics in a progressive cooling situation, this is unlikely to be true for all real situations in which temperature fluctuates Nakai[3] concluded from the stable oxygen isotopic measurements of water in Lake Vanda that most of the present lake waters originate from a freshwater source It is probable that in the initial stages of formation of the lake the isolated seawater evaporated to form salt deposits Since Lake Vanda has no outlet, these salts in the Wright Valley may have been washed into the lake at persistently low temperatures when there was a paucity of liquid water In these conditions Mg^{2+} is 3 to 4 times more soluble than K^+, and $MgCl_2$ $12H_2O$, $MgCl_2$ $8H_2O$ and $MgCl_2$ $6H_2O$ are stable from $-34\,°C$ to $-17\,°C$, from $-17\,°C$ to $-3\,°C$ and from $-3\,°C$ to $117\,°C$, respectively A temperature rise causes a phase change to a less heavily hydrated salt and the liberation of its own water of crystallisation in which the salt dissolves readily On the other hand, deposited potassium chloride requires a much slower diffusion of liquid water into the region of the crystal before solution can occur This mechanism may help to explain the enrichment of Mg^{2+} relative to K^+ during the accumulation of freshwater in the lake

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Measurement of the frequency of the methane-stabilised laser at 3.39 μm and of the R(32) transition of CO_2 at 10.17 μm

ACCURATE, infrared, frequency and wavelength measurements now constitute the best means of determining the speed of light, and provide a link between the standards of length and time We measured the frequency of the methane-stabilised He–Ne laser at 3 39 μm, and found good agreement with the result obtained by Evenson *et al*[1] The methane frequency was related to the caesium standard by using both the R(12) transition of CO_2 at 9 3 μm, which has already been measured[2], and the R(32) transition of CO_2 at 10 17 μm

Our result for the $F_2(2)$ component[3] of the P(7) transition of the v_3 band of methane is

$$f_{CH_4} = 88,376,181,610 \pm 70 \text{ kHz},$$

where f_{CH_4} and its uncertainty are rounded to the nearest 10 kHz

The frequency which we obtained for the CO_2 R(32) transition at 10 17 μm is

$$f_{R(32)} = 29,477,160,862 \pm 21 \text{ kHz}$$

The uncertainties consist of the root sum of squares of the random and systematic components which are, respectively, ($\pm$ parts in 10^{10}) 2 1 and 7 4 for f_{CH_4} and 1 8 and 6 8 for $f_{R(32)}$

The laser frequencies were measured by using the harmonic mixing properties of metal–insulator–metal point contact diodes (see ref 2) The frequency measurements were carried out in two stages

$$f'_{R(32)} = f'_{R(12)} - 3f_{HCN} - 27 \text{ GHz} + \Delta f_{beat} \quad (1)$$
$$f_{3\ 39} = 3f'_{R(32)} - 55 \text{ GHz} + \Delta f_{beat}, \quad (2)$$

where $\quad f_{3\ 39} = f'_{CH_4} - 50 \text{ MHz}$

The primes indicate that the frequencies are those of stabilised lasers rather than those of the reference transitions The parameter f_{HCN} is the frequency of an 891 GHz, HCN laser[2], and $f_{3\ 39}$ is that of a 40 mW, single mode He–Ne laser (offset locked to the methane-stabilised laser) introduced to provide sufficient power for harmonic mixing

In stage (1) 39 blocks of 10 readings were obtained on three days over a 12 week period The standard error of the mean, σ_m, of the 39 averages was 1 3 parts in 10^{10} In stage (2) about 60 readings per day were obtained on five days over a three week period For each day's results, σ_m was approximately 1 1 parts in 10^{10} and, because of the larger uncertainty of the correction applied to each daily mean for the background slope, there was no further improvement in the random uncertainty (In each of the two stages the standard deviation of a single reading was about 1 part in 10^9 and the day-to-day variation in the mean corrected result was about ± 3 parts in 10^{10}) The total random uncertainties (parts in 10^{10}) for our $f_{R(32)}$ and f_{CH_4} results, 1 8 and 2 1, respectively, arise from combining 1 3 from the base CO_2 R(12) measurement[2] with 1 3 from stage (1) for $f_{R(32)}$ and with the further 1 1 from stage (2) for f_{CH_4}

The two CO_2 lasers were each stabilised by controlling the frequency with reference to the saturated-absorption dip observed using the 4 3 μm fluorescence[4] from an external CO_2 gas cell Details of these stabilised lasers will be published

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Table 1 Systematic uncertainties ($\pm$ parts in 10^{10})

Source	Contributions to $f_{R(32)}$ and to f_{CH_4}	
Rubidium standard against caesium	0 3	
Counting errors	2	
Room temperature drift	2	
HCN laser asymmetry*	2	
CO_2 R(12) laser reproducibility	3	
CO_2 R(12) transfer (quadrature sum)†	4 6	
CO_2 R(32) laser reproducibility	3	3
CO_2 R(32) offset from transition centre	4	
CH_4 stabilised laser reproducibility		4
CH_4 offset from transition centre		3
Quadrature sum	6 8	7 4

*An allowance for any difference between the frequency measured by time-averaged counting and that obtained by observing a spectrum analyser display

†The CO_2 R(12) transfer sum combined with a further 4 parts in 10^{10} to allow for offset from the transition centre gives our present estimate of systematic uncertainty (6 1 parts in 10^{10} against the previous 8 3) for the CO_2 R(12) measurement (see ref 2) (The allowances for HCN laser asymmetry and CO_2 laser reproducibility have been reduced as a result of further experimental investigation)

The methane-stabilised He–Ne laser[5] had a d c -excited gain tube which produced maximum power output at a frequency 100 MHz lower than the methane saturated-absorption feature This was a small peak in the output power produced by an intracavity absorption cell 100 mm long, filled to 40 mTorr This cell resulted in a feature only 0 2 % as high as the maximum power output The first-derivative technique used for locking necessitated a correction of about 60 kHz (6 8 parts in 10^{10}) for the background slope The main contributions to systematic uncertainty, including those now considered appropriate to our previous CO_2 R(12) result, are summarised in Table 1

This measurement of the methane frequency agrees well with that of Evenson *et al* (88,376,181,627$\pm$50 kHz) It is of interest that their value, together with four independent measurements of the wavelength, was used in June 1973 by the Comité Consultatif pour la Définition du Mètre to arrive at a recommended value for the speed of light[6] with an uncertainty of $\pm$4 parts in 10^9 Although this uncertainty stems mainly from the determinations of wavelength, and is much larger than the quoted uncertainty of the National Bureau of Standards (NBS) frequency measurements, the good agreement of our result with theirs reduces the chance that some unsuspected error remains, the more so because our experimental method differed considerably in detail, though not in broad principle, from that of the NBS group

The systematic uncertainties associated with the methane-stabilised laser are much reduced in an improved type now being tested, and further frequency measurements will be made These results were communicated (with a provisional assessment of the uncertainties) to the Conference on Precision Electromagnetic Measurements, in London on July 4, 1974

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Capillary rise in porous media

Usually the porespace in a porous medium is visualised as a more or less complicated assembly of isolated or interconnected capillaries, and such porespace models are used in describing transport phenomena in porous media If capillary rise of a (wetting) liquid in a homogeneous irregular packing of rotund particles is considered, however, it seems that none of the existing porespace models is capable of explaining, not even qualitatively, the observed phenomena[1]

This may be illustrated by presenting two experiments, the capillary rise of glycol and of *n*-heptane, in a homogeneous packing of polystyrene spheres When equilibrium has been reached, it can be observed that for glycol there is a sharp front between the completely saturated part of the porous medium and the dry part of the porous medium, that is, no saturation gradient is displayed by the system glycol–polystyrene In the case of heptane a wide saturation gradient is observed in the equilibrium situation (Fig 1) Within the conventional porespace models the former result suggests a model of equally sized cylindrical capillaries, whereas the latter result suggests a model of capillaries of varying size It can therefore be seen that these two results lead to inconsistent pictures of what is physically the same packing

Numerous experiments show that the contact angle, θ seemingly has some bearing on this anomaly The contact angle is a notorious parameter to determine for flat smooth surfaces, and for particles (such as beads or sand) a direct determination is almost impossible From the work of Zisman[2] and others it may be concluded that $\theta = 0°$ for heptane–polystyrene and $25°$–$50°$ for glycol–polystyrene Capillary rise of water in sand yields a wide saturation gradient at equilibrium when the sand has been cleaned by heat treatment No saturation gradient (and a lower capillary rise) is observed when the sand is slightly contaminated Apparently, a wide saturation gradient corresponds to θ being approximately zero and the absence of a saturation gradient corresponds to θ being above some critical value

We can explain the correlation between θ and the width of the saturation gradient for irregular sphere packings The basic
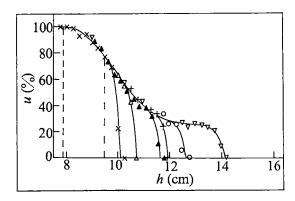
Fig 1 Capillary rise of *n*-heptane in a homogeneous packing of polystyrene spheres (particle size 175–250 µm), h is the height above the free liquid surface and u is the volume per cent porespace filled with liquid The results are from two different experiments with two different packings The exact form of the saturation gradient at equilibrium is somewhat uncertain because, at the times involved (10–40 d), secondary phenomena (capillary condensation, temperature fluctuations) become significant The homogeneity of the packing and the liquid saturation is measured using an X-ray absorption technique[5] The absence of a saturation gradient, as for glycol, means, operationally, that the saturation gradient is smaller than 1 mm First experiment after $\times$, 4 h, ▲, 40 h Second experiment after — — — — , 0 5 h, — — — — , 2 5 h, △, 5 h, +, 50 h, ○, 122 h, ▽, 480 h

reason why all porespace models fail is that they entail the presence of a large number of isolated menisci in the 'pores' of the porous medium This is not the case During capillary rise the liquid front consists of one continuous meniscus From this it necessarily follows that the meniscus consists of concave parts separated by anticlastic parts

Anticlastic means that the two main radii of curvature are of opposite sign We shall use the term 'meniscus' as an abbreviation for the 'concave or anticlastic part of the liquid-vapour interphase'

The anticlastic parts or α menisci lie between the adjacent parts of two spheres The concave parts or γ menisci are contained by three or more spheres and three or more α menisci The mechanism of capillary rise is governed by a continuous merging and generation of α and γ menisci in the porespace, consisting of 'cavities' connected by 'windows' Continuity of transport is sustained by two intersupporting mechanisms First, the filling of windows by merging α menisci, (the closure of the window generates new α menisci Second, the filling of cavities by merging γ menisci (the closure of cavities generates new α and γ menisci) Primarily, capillary rise is dependent on the first mechanism—that is, both α and γ menisci contribute to the advancement of the liquid front, but the α menisci are the leading agents The merging α menisci in a window may generate enough new α menisci to maintain continuity of transport by α menisci only The γ menisci are necessary to fill the cavities but they cannot, by themselves alone, maintain continuity of transport, because, on average, the number of γ menisci necessary to close a cavity is larger than the number of new γ menisci generated by the closing cavity

The maximum possible amount of capillary rise is determined by the curvature at which two α menisci merge in the smallest window of the packing, that is in any window between three contiguous spheres For $\theta = 0$ this curvature is given by $Cd = 0\ 11$, where C is the sum of the inverse radii of curvature and d is the particle diameter[4]

The curvature calculated from the observed maximum capillary rise (that is, the top of the saturation gradient, about 14 cm in Fig 1) of various liquids in glass beads and polystyrene spheres yielded values of Cd in the range $0\ 10$–$0\ 12$

As a first approximation the saturation gradient at equilibrium after capillary rise is a function of part of the window-size distribution In an irregular sphere packing there are a large number of smallest windows[3], so it is possible that no saturation gradient is observed when the α menisci filling the smallest windows can maintain continuity of transport, together with the γ menisci Quantitative data on the curvature of α and γ menisci and the statistical properties of particle packings are required to predict the width of the saturation gradient Even for the most simple cases these values are not—or are hardly—known It is known, however, that with increasing θ the curvature of the α menisci decreases more rapidly than that of the γ menisci[4,6] This implies that at higher values of θ the contribution of the γ menisci becomes more important, and thus that less α menisci are necessary to maintain continuity of transport Therefore, the hypothesis is put forward that the width of the saturation gradient decreases with increasing θ Above some critical value of θ the maximum capillary rise is determined solely by the filling of the smallest windows by merging α menisci, and thus no saturation gradient is observed

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The modulation contrast microscope

THE 'modulation contrast microscope' is a new type of imaging system with which transparent or phase objects can be visualised with a clarity and contrast equal to, or greater than, that of other light microscope systems A bright field microscope is readily converted into a modulation contrast microscope by the addition of an aperture slit before the condenser and a modulator after the objective in a plane conjugate to the slit, the Fourier plane (Fig 1) The modulation contrast microscope makes visible phase gradients, optical gradients and surface slopes
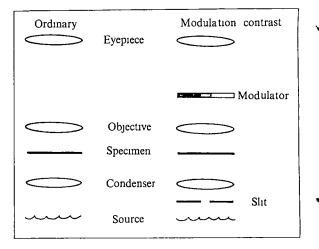
Fig 1 Modulation contrast microscope schematic compared with bright field microscope

Images viewed under modulation contrast are unobscured by phase objects above or below the plane of focus (optical sectioning) A transparent specimen, the human epithelial cheek cell, when viewed under a $\times 40$ objective, NA0 65 (Fig 2a), reveals nucleus and cytoplasmic granules and cell edges as a three-dimensional image Focused at another level, the cell membrane's corrugated folds and adhering bacteria are resolved (Fig 2b)
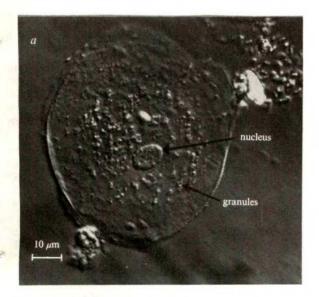
Modulation contrast is most sensitive to gradients normal to the slit orientation, the system is directional, as indicated by the direction of the optical shadowing at the boundaries of the nucleus in Fig 2a The image is shadowed in the direction of gradient detection, bright on one side and dark on the other

The resolution of the modulation contrast microscope is shown to be comparable to bright field and interference contrast A test object for the $\times 40$ objective is the diatom, *Pleurosigma angulatum*, the pore spacing of which is at its resolution limit These pores are clearly delineated by modulation contrast

The principles underlying the design of the modulation contrast microscope may be explained by describing the operation of the modulator, a specially designed filter in the Fourier plane An elementary modulator (Fig 3a) has three zones of different transmittances such that there is a dark region, T_D, a grey region, T_G, and a bright region, T_B, with values such that

$$T_B > T_G > T_D$$

For most objects, transmittance values approximating $T_B = 100\%$, $T_G = 15\%$ and $T_D = 1\%$ provide satisfactory images of a wide range of gradients The modulator is placed in the Fourier plane beyond the objective where the luminous image
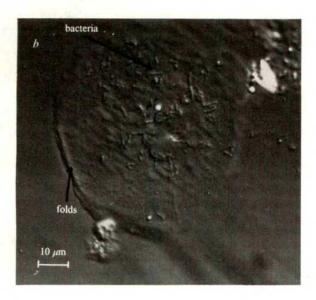
Fig. 2 Human epithelial (cheek) cells seen under modulation contrast with ×40 objective, demonstrating optical sectioning. a, Nucleus and cytoplasmic granules seen; b, cell surface without obscuring by lower structures; cell membrane folds and adhering bacteria seen.

of the slit falls on and coincides with the grey region of the modulator. A schematic diagram (Fig. 4) shows that light passing through the specimen where there are no gradients must pass through the grey region of the modulator where it is reduced to 15% of its former intensity and proceeds to the image plane, producing, for the most part, a grey background illumination. Light passing through positive gradients is refracted, in whole or in part, into the bright region, producing brighter features in the microscopic image; negative slopes direct rays which are attenuated by the dark region to produce darker features in the image. This type of modulation leads to an illusion of three-dimensionality which does not represent the geometric object, but the variation in optical density. The modulator affects only light amplitude and does not cause phase changes. Measurements on a Michelson interferometer confirmed phase changes, if any, as below $\lambda/20$.

Similarly, the three zones may be regions with different colours, but with transmittance ratios as in a neutral density modulator. Each optical gradient then produces intensities as before, but in colour; similar gradients will have similar colours.

Resolution is dependent on the exit pupil of the Fourier plane which is approximately the bright region of the modulator. In a symmetrical system, where the grey region is on the optic axis, the resolution approaches λ/NA_{obj}, equivalent to axial illumination as described by Abbe. To maximise resolution, the grey region is offset to the edge of the exit pupil; then the dark side of the modulator is outside the exit pupil (Fig. 3b). To match the offset modulator, the aperture slit is also offset. In these conditions, resolution approaches $\lambda/2NA_{obj}$, that of oblique illumination, and is maximised.

An objective containing a modulator can be used successfully for bright field observation of stained specimens by removing the slit aperture.

The theory underlying modulation contrast microscopy shows that phase gradients in the object plane distribute zero order amplitude across the entire exit pupil. The modulator preserves the sign of the gradient, processing differently the zero orders of opposite gradients, and the image contrast and visibility is relative to background intensity, set, primarily by grey region modulator transmittance.

A plane wave entering the system in complex notation, is e^{-ikz} where k, the wave constant in air, is $2\pi/\lambda$ and z is the direction of the optic axis. The wave constant for a ray passing through the object is then $k' = (n_o - n_m)k$ and $z = x\tan\alpha$ where α is the gradient. Let $\varphi = k'\alpha$, then $e^{-i\varphi X}$ describes the wave leaving the gradient. The object function in the Fourier integral is then $e^{-i\varphi X}$ and the maximum energy for different gradients is distributed across the Fourier plane.

When $e^{-i\varphi X}$ represents the gradient in any phase object, then the amplitude function in the Fourier plane is
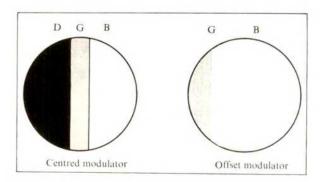
$$U_{(\theta)} \sim \int e^{-i\varphi X} e^{-i\theta X}/\mathrm{d}X \qquad (1)$$

where θ is the angular dimension in the Fourier plane and φ represents the phase gradient in the object and X is the dimension in the object plane. The amplitude distribution across the Fourier plane is

$$U_{(\theta)} \sim \sin(\theta \pm \varphi)/(\theta \pm \varphi) \qquad (2)$$

For simplicity, the width of the object has been omitted. The maximum amplitude in the Fourier plane for any phase gradient in the object is not at zero angular position, but at $\theta = \pm\varphi$. In the absence of phase gradients, the maximum amplitude would be located at $\theta = 0$, or at the conjugate of the source aperture. In practice, the offset modulator is used. Then, for an incident angle of illumination, γ, φ is replaced by $\gamma \pm \varphi$. For gradients with $\varphi \neq 0$, the zero order amplitude is shifted out of the grey region and is modulated above or below this

Fig. 3 Elementary modulator design with three transmittance regions within exit pupil. a, with centred grey region; b, with offset grey region to maximise resolution.

Centred modulator

Offset modulator
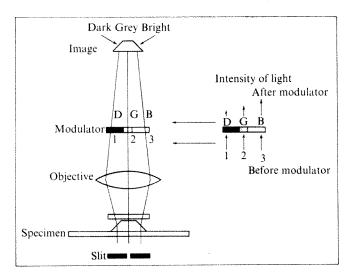
Fig. 4 Schematic diagram indicating the regions of the modulator which process light from the phase gradients in the object to form an image of contrast. No phase changes are introduced by the modulator.

value by modulator action, producing image contrast. This type of modulation leads to a three-dimensional appearance in the image plane.

Our experimental results are consistent with equation (2). Phase gradients shift the maximum intensity peak (zero order) of the diffraction pattern of a specimen feature away from the grey region and distribute the zero order (as well as higher orders) throughout the Fourier plane. It has been said that the zero order of the pattern carries no information[1]. We have shown this to be untrue. The location of the zero order in the Fourier plane is a measure of the optical gradient. Its intensity, with or without modulator processing, controls the intensity or contrast in the final image; without a modulator, there is no contrast between the gradients and the background. Abbe[2] developed the diffraction theory of image formation for amplitude objects: Zernike[3] extended the theory to phase objects. Dodd[4] discusses his schlieren microscope, stating that gradients shift zero orders. We have carried the imaging theory further by preserving the sign of phase gradients while maintaining full resolution; the theory is described in detail elsewhere[5].

We thank Michael Brenowitz for assistance. Bernard Friedman and the Nikon Instrument Group of Ehrenreich Photo-Optical, Inc. for the use of their instruments and James Rlazarian for assistance in preparing Fig. 2.

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Early domesticated sorghum from Central Sudan

LARGE quantities of carbonised *Sorghum bicolor* (L.) Moench grains, spikelets (Fig. 1*a*) and inflorescence fragments sorted from about 2 foot[3] of charred material found in a storage pit at Jebel et Tomat (13° 36′N, 32° 34′E) in Central Sudan (Fig. 2), and small amounts of carbonised sorghum found in eleven levels of the midden excavated there, suggest that sorghum

was the staple grain of people who inhabited the site. The date of 245±60 AD (UCLA 1874M) was obtained from a concentration of carbonised plant remains in the floor of the pit, which was dug into the dark clay loam on which the midden rests (Fig. 3) probably at about the same time as the accumulation of the middle or beginning of the upper unit of the midden. The remains of wickerwork matting and many fragments of thick stalks of cereal grass suggest that the pit may have been a silo lined with stalks and mats. If so, it is not dissimilar to the pits made today in the area for storing grain.

Most of the carbonised sorghum grains examined had 'popped' or become distorted in shape in the process of carbonisation, but sufficient specimens were well enough preserved to indicate the approximate size and shape of the grain. Undistorted carbonised grains were 3–3.4 mm long and 2.3–2.9 mm wide (Fig. 1*b*).

Jebel et Tomat is an inselberg of granite, gneiss and basic igneous rocks. Radiocarbon dates of settlement debris excavated on the jebel indicate the site was occupied from about 40 BC to 430 AD. Charcoals from the excavations identified by the Jodrell Laboratory, Kew, as *Acacia* sp., *Salvadora persica*, *Ziziphus* sp. (probably *Z. spina-christi*), and *Ficus* sp., are evidence that the region was about as arid during the period of midden accumulation as it is today (G. Wickens, personal communication). The abundance of opal phytoliths in the midden further indicates that there was then no scarcity of grass and reeds in the immediate vicinity of the site[1].

The settlement debris suggests a sizeable population, because it covers more than 12 acres and because 80–120 cm, on average, is the depth of the occupation midden, which consists of a grey-brown sandy loam, stony in the upper levels and more compact in the lower but lacking any clear evidence of disconformity. Scattered through the midden were potsherds, grindstone fragments, flaked stone debris and tools, pottery labrets and anthropomorphic and zoomorphic figurines of baked clay, together with bones from food waste, shells, charcoals and fragmentary plant remains. The prehistoric population seems to have been negroid, similar to that from Jebel Moya[2] and, although they were still using stone for cutting equipment and ground axes, a trace of iron is present, together with beads of blue faience and zeolite that were probably obtained through trade.

On the basis of colour, consistency and small changes in lithology, the deposit has been divided into three units the accumulation of which could have followed brief periods when the site may have been abandoned (Fig. 4). Pits for storage and other uses as well as graves dug by the later occupants into the earlier beds have been the cause of no small amount of disturbance. A run of five radiocarbon dates (Table 1) was obtained and these reflect this disturbance since the three lower ones are reversed. The dates obtained, however, confirm the evidence of the cultural remains (pottery and stone tools) that the accumulation represents a single, continuing occupation of not particularly long duration at the beginning of the first millennium AD and so broadly contemporary with Meroitic civilisation in Nubia. The well known site of Jebel Moya, some 60 km to the east and excavated in 1910–14 by Sir Henry Wellcome[3], is believed to be in part contemporary with Jebel et Tomat since the pottery traditions from both are similar.

The economy at both Jebel et Tomat and Jebel Moya was one of mixed farming. At Jebel et Tomat most of the meat was obtained from domestic cattle and sheep/goats, supplemented by fishing, fowling and the hunting of gazelle and small antelope; *Pila* shells were also collected, either for food or bait.

Examination of the carbonised plant material led to the following conclusions:

(1) The sorghum from the storage pit was fully domesticated, domestication being indicated by the loss of anatomical features which facilitate dispersal of seed by natural agents. Spikelets bearing grain in the storage pit were still attached to branchlets of the inflorescence or rachis fragments. If the sorghum were not domesticated, the spikelets bearing the grain
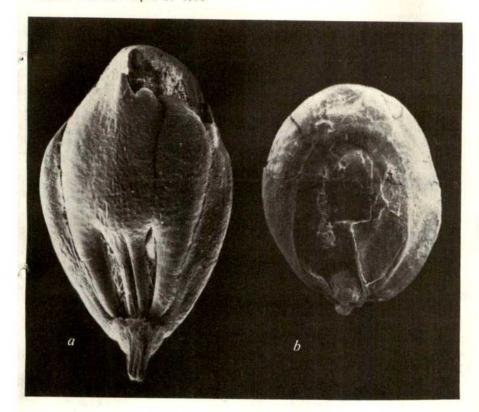
Fig. 1 *a*, Electron micrograph (×12) of spikelet of *Sorghum bicolor* (L.) Moench race bicolor, from the storage pit excavated at Jebel et Tomat. The top of the grain expanded in the process of carbonisation. Attachment of branchlet of the inflorescence indicates that this is domesticated sorghum. Photo: J. N. Brunken, *b*, Electron micrograph (×12) of a carbonised grain of *Sorghum bicolor* race bicolor from the Jebel et Tomat storage pit. This view shows the embryo. Photo: J. N. Brunken.

would have been separated from the supporting branchlets by formation of a zone of abscission at the base of the glumes which facilitates dispersal of seed.

(2) The sorghum from the storage pit belongs to the race bicolor of *Sorghum bicolor*. This is indicated by the fact that the grain is fully enclosed by the glumes[1]. The size and shape of the carbonised grain and spikelets are well within the range of variation of cultivated sorghum of race bicolor grown in many parts of Africa at present.

(3) There is no readily apparent difference in the shape and size of sorghum grains found in various levels of the midden excavated at Jebel et Tomat when compared with sorghum from the storage pit.

(4) Sorghum grain preserved in the storage pit and midden is smaller and more 'primitive' in morphology than that grown in the area at the present. There are several possible explanations for the apparent difference.

First *Sorghum bicolor* race bicolor preserved in the storage pit at Jebel et Tomat may have been only one of several kinds of sorghum grown in the area 1,700–1,900 yr ago. Larger-grained sorghum may have been stored in other pits which simply were not preserved.

Second *Sorghum bicolor* race bicolor may represent the only kind of sorghum grown at Jebel et Tomat but may not be an indication of how much progress had been made in selection of highly derived sorghums in other parts of Africa by 245±60 AD.

Third sorghum from Jebel et Tomat may indicate the progress made by 1700 BP in the course of selection of highly derived sorghums now grown in the north-eastern part of the African savanna. If so, the selection and change resulting in the greater size and the morphological characteristics of sorghums now typical of much of Central and southern Sudan may have occurred in the last 1,700 yr.

Table 1 Radiocarbon dates from the prehistoric settlements at Jebel et Tomat and Jebel Moya

Sample	Jebel et Tomat excavation	Location	depth (cm)	¹⁴C date (yr b.p.)	AD/BC date
UCLA1864	T.T.1	Upper unit A/1	40–50	1,600±80	350±80 AD
UCLA1874I	T.T.1	Middle unit A/0	60–70	1,930±60	20±60 AD
UCLA1874J	T.T.1	Middle unit A/1	70–80	1,770±60	180±60 AD
UCLA1874K	T.T.1	Middle unit A/1	80–90	1,735±60	215±60 AD
UCLA1874M	T.T.1	Upper/Middle Unit A/O (Storage pit)	110–120	1,705±60	245±60 AD
SUA-67	Soil pit on periphery of midden	Dark clay loam	40–80	4,540±200	2,590±20 BC
	Jebel Moya				
UCLA1874D	Test pit: western perimeter	Compact light brown gritty sandy loam (unit 3)	80–90	4,200±80	2,250±80 BC
UCLA1874E	Test pit: western perimeter	Compact light brown gritty sandy loam (unit 3)	90–100	4,200±80	2,250±80 BC

The above dates are on charcoal excepting SUA-67 which is on shell.
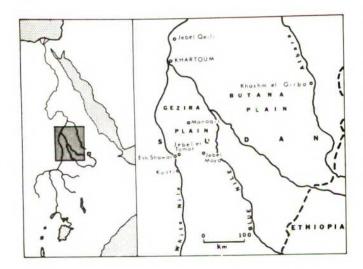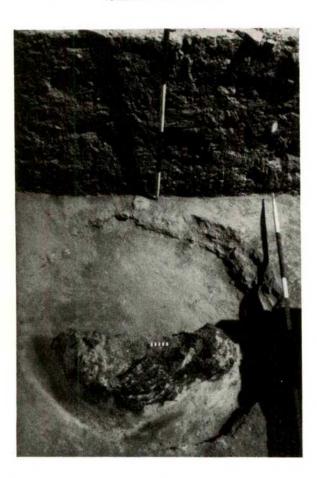
Fig. 2 Map of the area and the sites referred to in the text.

In the Jebel et Tomat excavations no evidence of dwelling or other structures was found, so these are most likely to have been built of temporary materials—grass or mats and poles—and to have been either easily transported (if the population was nomadic) or rebuilt every year, in either case without leaving any permanent record. Considering all the evidence available on the palaeoecology[5] and economy of Jebel et Tomat and other sites in central Sudan, it seems most probable that the Jebel et Tomat population of the early first millennium was transhumant, living along the Nile from mid-January to June

Fig. 3 Lower part of the storage pit dug into the dark clay loam, showing one half of the concentration of carbonised plant remains. Photo: J. D. C.

when they moved east with their herds to Jebel et Tomat to graze the Manaqil ridge and the surrounding grasslands. Sorghum and, no doubt, other crops such as dukhn (*Pennisetum americanum*) and lubia (*Vigna* supp.) were planted in the summer growing season and reaped in November–December after which the people moved west to the Nile again. Such a pattern is not unlike that followed today.

The evidence from Jebel et Tomat is not alone in indicating the importance of sorghum in economies of Central Sudan in the early part of the present era. Sorghum, provisionally identified as the bicolor race (Eklass Abd El Bari, personal communication) has been found at Handal, a somewhat later site in Nubia. Also from Nubia comes a bouquet of sorghum
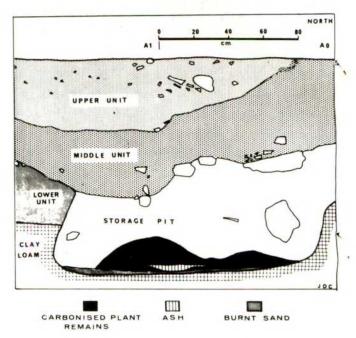
Fig. 4 North-South section across centre of storage pit containing carbonised Sorghum, Jebel et Tomat; excavation T.T.1.

inflorescences of race bicolor (identification verified by J. R. Harlan and J. M. J. de Wet, personal communication) found in a (?)ritual pit dug into a late Meroitic pavement at Qasr Ibrim[6].

In the engraving of the Meroitic King Sherkarer (12–17 AD) at Jebel Qeili in the southern Butana, about 60 km north of Khartoum, heads of sorghum appear to be represented (ref. 7 pages 51 and 159) and millet, presumably sorghum, is referred to in particular by the geographer Strabo following his visit to Egypt in 25–24 BC. He stated: "The Ethiopians live on millet and barley from which they also make a drink . . ." He goes on to say "But some have grass as food, as also tender twigs, lotus and reed roots; and they use meats, blood, milk and cheese"[8]. This suggests that cereal cultivation had not yet become universal on the Nubian Nile and was probably subsidiary in importance to stock herding, as it is today.

Evidence cited above suggests that sorghum was a major crop of the Meroitic Kingdom, but how much earlier it was domesticated remains to be shown. Both Jebel et Tomat and Jebel Moya shared a number of cultural traits in common with what has been called the Butana Industry from Khashm et Girba in the Atbara drainage some 340 km to the east north-east where there are similar substantial midden accumulations at six sites. Site N125 is not yet excavated, but a charcoal sample (Tx445) from *in situ* in a test pit gave a result of 4,410±90 b.p. (2460 BC), in general agreement with the earlier dates at Jebel et Tomat and Jebel Moya[9]. All these sites belong to a single long lived cultural tradition that had its beginnings in the

early third millennium BC in the Khartoum Neolithic and that, in its later stage, shows a number of traits in common with Meroitic culture in the centuries immediately before and following the beginning of the present era. It is not inappropriate to term this the "Jebel Moya tradition" and, when a search for plant remains from the third millennium settlements is carried out, it would not be unexpected if an early form of domestic sorghum were found to be present.

We thank Rainer Berger for the radiocarbon dates; Gerald Wickens for charcoal identifications; Donald Adamson for identification of sponge spicules in the Jebel et Tomat pottery; Eklass Abd El Bari for information on her provisional identification of the Handal sorghum; and Peter Shinnie for information on the Nubian sites yielding sorghum. We also thank Andrew Smith, Daniel Stiles and Kenneth Williamson who took part in our excavations and were responsible for analysis of cultural and faunal material; Martin Williams and Donald Adamson for their help and close collaboration in the field; the Sudan Department of Antiquities and members of the Departments of Botany, Geology and Archaeology at the University of Khartoum; and Betty Clark for secretarial assistance. The archaeological investigation was made possible by grants from the British Academy and the Ford Foundation.

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First direct evidence of life under Antarctic shelf ice

A TOPIC of current interest to Antarctic marine biologists is the possibility of a biome at considerable distances from the open sea under the vast, permanent ice shelves that fringe areas of Antarctica. Such biological information is one of the aims of the present Ross Ice Shelf project[1], an attempt to drill through ice 500 m thick at 82°30'S, 166°00'W, a site 450 km from the ice front in the Ross Sea. We have already obtained, under unusual circumstances, direct evidence of a biome under shelf ice at least 100 km from the open sea.

During the 1973–74 austral summer we carried out a limnological survey between latitudes 70°48'S and 71°20'S on the west coast of Alexander Island (Fig. 1). King George VI Sound, which separates the island from the continent (Palmer Land), is covered by shelf ice 100–500 m thick[2]. The shelf ice receives glacier ice from both Palmer Land and Alexander Island. The resultant force is towards the northwest and rows of pressure ridges, 10–15 m high, lie along the west coast of Alexander Island in this latitude.

We caught four specimens of a common Antarctic marine benthic fish, *Trematomus bernacchii*, in a large (5 × 4 km) proglacial lake, lying in Ablation Valley (70°49'S, 68°25'W), (Fig. 2). The lake is over 117 m deep and has a permanent ice cover, 4.0–4.5 m thick in winter, 2.5–3.0 m thick in summer. The annual mean air temperature is approximately −9 °C. Ice
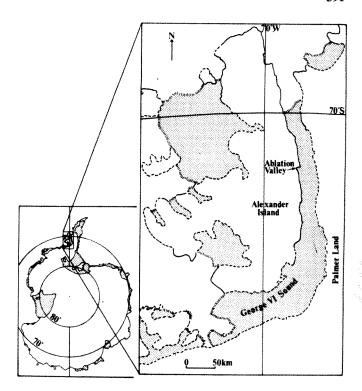
Fig. 1 Outline map of Alexander Island and its position in Antarctica. Ice coastline definitive/approximate is shown by continuous/dotted line. Ice shelf, stippled area. Front of ice shelf, dashed area.

from the Sound pushes into the lake along the 70 m depth contour for 3.4 km. Salinity measurements revealed that the top 55 m of water were fresh (0.1–1.0‰) but below 66.5 m the salinity was 32‰. The halocline was very steep and between 66.00 and 66.25 m the salinity rose from 18 to 31.5‰. The lake surface moved up and down with an irregular diurnal tidal rhythm. Variation in salinity profile with tidal movement indicated that the saline layer was in direct contact with the seawater of the Sound. The seawater extended for 4.0 km into the lake. Approximately 12% of available light entered the lake during the summer and we estimate that less than 0.05% reached the seawater layer. Heat gain by the water is restricted by the high latent thermal capacity of the lake and Sound ice, and the seawater. Temperatures remained almost constant throughout the period of investigation, the profile ranging from +0.10 °C to −1.60 °C (seawater). The oxygen content of the water was between 100 and 90% saturation in the freshwater layers, falling to 53% saturation in the seawater layer.

The fish were caught in a trap lying in 70 m of water. Seal meat was used as bait. The statistics of the fish are recorded in Table 1. The fish were in good condition although the weight–length ratios were lower than those recorded by Hureau[3] and Wohlschlag[4] for specimens captured in the equally cold waters off Terre Adelie and in McMurdo Sound. Only one of the Ablation Valley specimens had mature gonads, a female of 12.60 cm standard length. Hureau[3] found that females of the Terre Adelie population became mature when 17.50 cm standard length on average (recorded range 16.20–20.00 cm), males when 14.50 cm standard length (range not given, smallest 13.00 cm). The contrast is sufficient to suggest that the individuals caught in Ablation Valley are of a smaller growth form.

The stomachs of the fish contained the remains of nektonic, planktonic and benthic organisms (Table 2). Muscle tissue attached to crustacean skeletal material indicated that the fish had fed recently. The freshwater layer of the lake has been colonised by a calanoid copepod, *Pseudoboeckella* sp. (average concentration recorded 0.5 individuals l⁻¹, including naupliar stages) but the remains removed from the fish were of different

Fig. 2 Aerial photograph of Ablation Valley. The lake occupies the whole of the wedge-shaped valley floor. King George VI Sound lies in the foreground. (Photography flown by the US Navy for the US Geological Survey).

and larger species. Phytoplankton production measurements by standard radiocarbon technique on 2 d at the height of the summer indicated that the amount of carbon fixed per day was about 60 mg m^{-2} for a zone 20 m deep. The only benthic vegetation seen during a SCUBA search covering about 10,000 m^2 in the freshwater zone was a thin film of algae on the occasional rock jutting out of a silt floor. We conclude that the productivity of the freshwater layer is probably extremely low and insufficient to support a fish population. Furthermore the fish were not adapted to freshwater and showed signs of acute distress when brought to the surface.

A cyclopoid copepod, as yet unidentified, was collected below 40 m depth from water of salinity range 0.6–32‰. The maximum concentration recorded was 0.06 individuals l^{-1}, although only 67 m of tubing were available for the sampling pump and this only just reached the marine layer. Although the cyclopoids were able to tolerate fresh/brackish waters they were presumably more numerous at greater depths.

T. bernacchii is a slow-growing fish of very sedentary habit. Our evidence suggests that there is a marine biome under the

Table 2 Gut contents

Stomach	Intestine
Copepoda, calanoid spp.	Copepoda, Calanoid spp.
Gnathiidae, whole larva	Copepoda, harpacticoid sp.
Fish eye lenses	Hydroid nematocysts
Fish scales, ctenoid	Tanaid sp.
Polychaetae spines (?)	Compound eyes, decapod,
Isopoda spp.	mysid or euphausid
	Porifera spicules

permanent ice of King George VI Sound, and in Ablation Valley which lies 100 km (northwards) and 334 km (southwards) from the open sea. There are two areas along the eastern shore of the Sound where the ice is fissured and seawater lies within a metre of the ice surface (Carse and Horse Points). Working through these 'holes' could provide further information on this remarkable biome.

We thank M. Macrae for the loan of his home-made fish-trap, A. Wheeler (British Museum) for identifying the fish, members of the Marine Section, British Antarctic Survey, for identifying some of the stomach contents, and Dr I. Everson for helpful comments.

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Lack of response to background colour in *Pieris brassicae* pupae reared on carotenoid-free diet

THE Large White butterfly (*Pieris brassicae* L.) is a toxic species[1], aposematic at all stages of its life-cycle. Probably for this reason, the pupa responds to the colour of its background in a somewhat different manner from that of many other Pierid species, displaying heterochromy (background contrasting) rather than homochromy (background matching). In this respect, there is some genetical variation between different strains (M. R., unpublished).

Gardiner[2] noted that in the Cambridge strain of this butterfly the diapausing pupa is invariably green, whereas most over-wintering cryptic British butterfly pupae are brown, and those of the summer broods green (ref. 3 and Allister Smith, unpublished). Non-diapausing pupae of the Cambridge strain are more labile and respond to background and other environmental cues, by colour changes, varying from silvery white, grey and green to brown, with more or less extensive blotching and freckling with dark brown or black. Non-diapausing pupae of *Papilio polyxenes asterias* Stoll are also more labile[4] than diapausing pupae, which are consistently brown in this species.

When caterpillars of *P. brassicae* (Cambridge strain) are reared on standard artificial diet lacking cabbage[5] (Table 1), virtually devoid of carotenoids (but not vitamin A), both the resulting diapausing or non-diapausing pupae are invariably turquoise-blue in colour, freckled with small black spots (Class 1 of the Oltmer melanisation scale)[6,7]. The cuticular keel and spiracles are white (Table 1). In such specimens (several thousands have been tested) there is no visible response to background, whether the caterpillars or prepupae are exposed to long or short hours of daylight, dim or bright illumination, or reared on smooth-surfaced artificial diet, or on rough-surfaced artificial diet in containers covered with: coarse dark

Table 1 Morphometric parameters of *T. bernacchii* from Ablation Valley

	Specimen			
	1	2	3	4
Sex	♀	♀	♀	♂
Size (cm)				
Total length	14.40	15.60	17.10	16.20
Standard length	12.60	13.60	14.80	14.00
Head length	3.90	4.23	4.87	4.26
Head width	2.80	3.01	3.32	*
Head depth	2.78	2.58	2.97	*
Snout length	1.02	1.22	1.22	1.20
Cheek height	0.36	0.34	0.33	0.34
Interorbital width	0.42	0.38	0.51	0.55
Orbit length	1.23	1.38	1.50	1.42
Eye diameter	1.03	1.22	1.08	1.18
Body depth	2.82	3.01	3.20	3.28
Pectoral fin length	3.05	2.93	2.89	3.13
Pelvic fin length	2.45	2.88	3.46	3.10
Weight (g)				
Total	36.85	43.30	60.90	47.05
Gonad	0.9412	0.0633	0.0610	0.0634
Fin-ray formula				
Dorsal	V, 36	V, 36	V, 37	V, 37
Anal	33	33	33	32
Pectoral	24	24	24	24

* Specimen died with opercula raised.

Table 1 Carotenoids in pupae of *P. brassicae* and their diets (selected examples*)

Food of larvae (not diapausing) at 70 °F	Number and colour of pupae and cuticle	Total carotenoids (μg per g dry weight)	Lutein (μg per g dry weight)	Carotenoids per insect (μg)
(1) Cabbage leaves Greyhound cultivar (2,750 μg per g carotenoids); pupating on wood	12 ♀ (whole) Silver-grey; melanin Class 5 or blacker; yellow cuticular keel	535.5	221.3 (41.6%)	17.75
	13 specimens; cuticle and epidermis only; same coloration	22.6	17.8 (78.8%)	0.24
(2) Artificial diet containing xanthophyll extract; (207 g per 100 g) pupating on wood	10 cuticles + epidermis 8 specimens, green; 2 specimens silver-grey; melanin Class 5 or blacker; yellow keel (indistinguishable from control, No. 1)	16.8	16.8 (100%)	0.15
(3) Artificial diet containing wheat germ; pupating on wood	9 specimens; Turquoise blue; melanin Class 1; white keel	1.2	ND	0.038
(4) Cabbage leaves 'January King' cultivar; pupating on white netting at sides of cage	6 specimens (whole) Green; melanin Class 1; yellow keel; indistinguishable from No. 5	247.6	139.2 (56.2%)	10.7
(5) Artificial diet containing wheat germ and xanthophyll extract from cabbage and β carotene pupating on white netting on sides of cage	6 specimens (whole); green; melanin Class 1; yellow keel; indistinguishable from control, No. 4	286.8	162.4 (56.6%)	8.6
(6) Artificial diet containing wheat germ; pupating on white netting on sides of cage	6 specimens (whole); turquoise-blue; melanin Class 1; white keel	13.7	13.7 (100%)	0.50
(7) Artificial diet containing xanthophyll extract; reared and pupating on glass	20 cuticles + epidermis; green; melanin Class 1; yellow keel	8.8	7.3 (83%)† 1.5 (5,6-mono-epoxy-α-carotene)	0.14

* Extraction methods were as described previously[19]. Other experiments with different cultivars and diets will be published elsewhere. The xanthophyll extract (NBC, Cleveland, Ohio) contained twelve or more different xanthophylls. Lutein made up only 1.4% of total carotenoids (5.4 mg per g dry weight), whereas 5, 6-mono-epoxy-α-carotene (69.7%) was the most abundant single xanthophyll in this extract. Wheat-germ, the overspill of manufactured Bemax, contained traces of four carotenoids—β carotene, lutein, zeaxanthin and flavoxanthin[18]. Hoffman LaRoche supplied β carotene, and Valadon and Mummery extracted the cabbage lutein.
† Contained 1.5 μg g^{-1} 5,6-mono-epoxy-α-carotene (17%) as well as 83% lutein.
ND, not determined.

brown hessian, in deep shade; smooth, light-coloured natural wood, in bright summer sunlight; rough white bath towelling in winter sunlight; or in glass jam jars in various conditions.

If lutein is added to the artificial diet (0.5 ml lutein extracted from African marigold added to 100 g standard diet, (see Table 1)), the resulting pupae are bright green instead of turquoise-blue, with a yellow cuticular keel and spiracles, and Class I black freckling. Lutein can then be shown to be present in the epidermis and cuticle (Table 1). Of 40 such pupae, several responded to the background and developed melanisation to varying degrees (Classes 1–5). Moreover, two were indistinguishable, both in colour and pattern, from the silvery grey and heavily blackened cabbage-fed controls (derived from the same egg batches) pupating alongside them on the wooden walls of the breeding cage and its glass front.

Relatively small (but not less than 1 μg) concentrations of carotenoids per individual seem to be required to mediate background response, since those pupae reared on white cabbage (131.9 μg g^{-1} carotenoids) contain only 1.58 μg of carotenoids per pupa, and are indistinguishable from green cabbage-reared specimens which each contain 12–19 μg per individual. Nevertheless, the 207 μg Xanthophyll per 100 g diet (Table 1) must be close to the lower limit which enables biliverdin and the carotenoids themselves to be partially excluded from the cuticle and epidermis[8,9], since only 6% of these lutein-reared pupae exactly matched the typical silvery grey and black coloration of the controls.

It has been generally accepted[8–11] that the colour and

pattern of *Pieris* and various other Lepidoptera pupae are controlled or modified by endocrine factors released from the anterior end of the body in response to a formidable and confusing array of sensory cues and biological mechanisms. It has been suggested that the melanisation of the cuticle and the distribution of ommochromes in the tissues of *P. brassicae* are controlled by different hormones from those which control the presence or distribution of bile pigments and the carotenoids themselves. Kayser and Angersbach[8], however, believe that the degree of melanisation and bile pigment content are regulated by the same hormonal factor. Our experiments indicate that at least in the case of the Cambridge strain of *P. brassicae*, carotenoids—possibly only lutein—function as a mediator, without which light cues associated with the insects' ability to respond to background do not operate. In the absence of dietary carotenoids the blue bile pigments[12,13] synthesised and released during the fifth larval instar and the 'sensitive' prepupal stage[8,14] remain in the haemolymph and epidermis and are neither re-routed nor degraded as in those pupae which turn silvery grey and black.

It is possible that carotenoids play some role in determining the colour modifications known to be associated with photoperiod, and characteristic of spring and summer broods of many Pierid butterflies[15–17]. It is also worth considering the influence of these substances in examples of circadian rhythms which, it has been suggested, could involve a photosensitive molecule participating in the clock mechanism[18]. In any case careful note should be taken of the carotenoid content of pupae and larvae

of Lepidoptera, whether reared on natural or artificial diet, which are used in experiments involving light cues.

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Mass production of marine algae in outdoor cultures

TWENTY-FIVE years ago the large scale culturing of unicellular algae was viewed with great enthusiasm as an alternative method for producing protein[1]. This hope diminished by the late 1960s when it seemed that the process was uneconomical because of a combination of technical problems, most notably the recovery of the algal product and its subsequent conversion to a human food and/or food supplement.

Quite recently though, there has been renewed interest in producing single-celled protein by mass culturing unicellular algae. This interest has been stimulated in part by the demonstration that algal systems can be used for both pollution control and protein production by directly recycling the nutrients in wastewater into the biomass of freshwater algae[2]. Recently, it has been shown that a marine counterpart, in which algae are grown on mixtures of wastewater and seawater, can serve the same roles with distinct advantages over freshwater algal cultures[3].

The algae are readily removed by utilising the microscopic organisms as the first link in marine food chains leading to multicomponent and integrated aquaculture systems[4].

As part of our efforts in developing the above process, we have been experimenting outdoors with continuous-flow algal pond cultures, trying to optimise biomass yields. We report here the results of long term experiments conducted during the past two years at Woods Hole, Massachusetts (May–October 1973) and Ft Pierce, Florida (May–October 1974).

The experiments were performed on the Woods Hole Oceanographic Institution dock and adjacent to the Harbor Branch Foundation Laboratory at Ft Pierce. The culture

Table 1 Effect of dilution rate on mean algal yield in two locations

	Mean algal yield (g dry weight $m^{-2} d^{-1}$)	
Dilution rate	Woods Hole (lat. 41°52'N)	Ft Pierce (lat. 27°28'N)
0.25	7.6 (2)	
0.50	12.4 (8)	18.0 (6)
0.75	13.3 (2)	15.3 (1)
1.00	12.4 (1)	23.6 (1)
1.50		0* (1)

Values in parentheses represent number of replicate experiments. Each experiment consisted of determining the steady-state algal yield at the stated dilution rate. The mean algal yields, where applicable were determined by averaging the data from the replicates. Variations from the means were less than $\pm 20\%$.
* Steady-state algal growth could not be maintained and the culture washed out.

units were two replicate ponds each of 2,000-l capacity, (2.3 m diameter and 50 cm deep) and lined with glass fibre. Secondarily treated domestic wastewater was obtained daily from local treatment facilities and stored in polyethylene tanks. Seawater was pumped continuously through 1-μm filters and blended with the wastewater into the ponds at the desired flow rates and mixture. The ponds were well mixed continuously with rotating arms and by recirculation through pumping so that they resembled completely mixed reactors.

Algal yield in a continuous culture is defined as the product of biomass concentration and flow rate on a per unit surface area basis. Thus by varying the dilution rate (fraction of volume turnover per day) in these studies from 0.25 to 1.50, we were able to determine the maximum attainable areal yields. For each dilution rate we establish relative steady-state conditions by maintaining a constant flow rate for 7–10 d.

A true steady state was impossible to establish because algal biomass concentrations were affected by daily variations in sunlight intensity and pond temperatures. Once relative steady-state conditions were established, however, there was no more than a $\pm 10\%$ daily change in biomass concentration. In both studies we added a mixture of 50% wastewater and 50% seawater to the ponds and continuously monitored inorganic nitrogen and phosphorus concentrations to ensure that nutrients never limited algal growth. We estimated algal biomass by measuring particulate carbon concentrations on a Perkin-Elmer 240 Elemental Analyzer; we then converted these values to ash-free dry weights by assuming that carbon comprises 45% of the total organic matter in algae, following experimental determination of this relationship.

Our results indicate that algal yields were relatively constant between dilution rates of 0.50 and 1.00 (average of 12.7 g dry weight $m^{-2} d^{-1}$ in the Woods Hole study and 19 g dry weight $m^{-2} d^{-1}$ in the Ft Pierce study), but were significantly reduced (7.6 g dry weight $m^{-2} d^{-1}$) at a dilution rate of 0.25 and were zero (cell washout) when the dilution rate was 1.50 (Table 1). The experiments at each location had to be spread over the May–October study periods; therefore, effects of seasonal changes in sunlight intensity and temperature were not eliminated as experimental variables. Some of the variations in the results, both in replicate experiments and at different dilution rates (Table 1), could be accounted for by changes in these environmental factors.

In both studies we did not innoculate the ponds with a particular algal species. Rather, when the ponds were first filled with wastewater–seawater mixtures, virtually a monoculture of a marine diatom species was quickly established from the indigenous algae present in the seawater. In the Woods Hole experiments *Phaeodactylum tricornutum* dominated during May and June, followed by *Amphiprora* sp. which prevailed until late August when *Amphora* sp.

became dominant. In late September through October *Amphiprora* sp. returned as the dominant alga. During the entire Ft Pierce experiment *Nitzchia closterium* was the main diatom.

We feel that our results are particularly noteworthy because they represent the first reported experiments on successful mass production of marine algae in fully continuous outdoor cultures that were maintained with little difficulty for the entire study periods indicated. In contrast, virtually all the data available on yields in outdoor cultures of marine and freshwater algae have been attained by intermittent collection in semicontinuous or batch cultures[5-8]. We have also shown the effects of geographical location on algal yields—the average yields at Ft Pierce were more than 50% greater than the average achieved at Woods Hole.

Our results compare favourably with yields of 10–20 g dry weight $m^{-2} d^{-1}$ that have been reported for freshwater algae in several European studies[5,6] and are considerably better than the 5–12 g dry weight $m^{-2} d^{-1}$ attained so far in marine mass cultures[7,8]. They are consistent with Ryther's original estimate[9] that, based on sunlight availability only, a maximum yield of about 27 g dry weight $m^{-2} d^{-1}$ can be sustained in a water body. It seems then not to be possible to achieve yields much above the values we have attained. As has been shown[9,10], these production values are comparable with those for the fastest growing agricultural crops such as sugar and rice.

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Genetic control of diploid-like meiosis in hexaploid tall fescue

ALLOPOLYPLOIDY has played a vital role in the evolution of plant species useful to man. Hybridisation between related species followed by chromosome doubling has given rise to some of our most important grain, forage and fibre crops. The successful establishment of a sexually reproducing polyploid would, however, depend on the integration of the constituent genomes into a meiotically and, hence, reproductively stable form which could be achieved only by means of a precise diploidising mechanism. In the case of bread-wheat it has been conclusively demonstrated that diploid-like meiotic behaviour is genetically controlled[1,2] and there is a strong indication of a similar control in other crop species, for example, hexaploid oats[3] and tetraploid cottons[4].
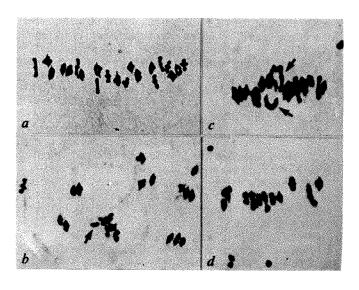
Tall fescue (*Festuca arundinacea* Schreb.) is an allohexaploid ($2n = 6x = 42$) comprising diploid genomes of three related diploid species of the *Lolium–Festuca* complex. That the three genomes are homoeologous is borne out by the fact that there is extensive, in some cases complete, pairing in the diploid, auto-

allotriploid and trispecific hybrids between the putative progenitors[5] and that some monosomics isolated by the author are fully fertile and morphologically indistinguishable from the sister euploid plants. Thus, although it contains three closely related homoeologous genomes, tall fescue regularly forms 21 bivalents between homologous chromosomes (Fig. 1*a*) indicating the possibility of genetic control of bivalent pairing. The evidence which strongly points to the presence of such a control is discussed here.

In a cross between the Algerian (*Bn 273*) and Israeli (*Bn 488*) ecotypes of tall fescue, all the five euploid ($2n = 42$) hybrids studied formed 21_{II}. The four euploids from the reciprocal cross (*Bn 488 × Bn 273*) also regularly formed 21_{II} (Fig. 1*a*). A sister plant which was a monosomic ($2n = 41$) numbered 90–64–7 formed some trivalents, quadrivalents (Fig. 1*b*), pentavalents and hexavalents, in addition to bivalents (Table 1). Another three monosomic plants of different genotypic background showed normal bivalent pairing. As all the euploid progeny from the cross *Bn 273 × Bn 488* regularly formed 21_{II} and the other monosomic lines had regular chromosome pairing (Table 1), it is reasonable to conclude that the missing chromosome in the monosomic 90–64–7 is critical for normal bivalent pairing. The consistent formation of 21_{II} in the parents of this cross and in all the euploid hybrids rules out the possibility of the occurrence of translocations in the monosomic 90–64–7. The occurrence of a hexavalent ,or a VI + IV or III, or V + IV or III, or IV + III, or 3 IV in the same cell would further discount the translocation hypothesis. The monosome itself was also sometimes involved in multivalent formation. It can therefore be inferred that multivalents in the monosomic 90–64–7 resulted from homoeologous pairing which was presumably caused by the removal of 'genetic regulator' on the missing chromosome. On this premise it seems that the genetic system controlling bivalent pairing in the hexaploid tall fescue is not effective in the hemizygous state, or at least, is not as effective as in the diploid state.

Clearly, in the other three bivalent-forming monosomics (Table 1), genetic information for the regulation of pairing was not located on the missing chromosome. For want of a full series of monosomics and nullisomics in tall fescue, however, it is not at present possible to conclude that the gene(s) system regulating bivalent pairing is solely confined to this particular chromosome involved in the monosomic 90–64–7.

The above inference receives substantial support from the

Fig. 1 *a–c*, Chromosome pairing in euploid and monosomic *F. arundinacea Bn 488 × Bn 273*; *a*, euploid hybrid with 21_{II}; *b*, monosomic 90–64–7 with 2_{IV} (arrowed) $+ 16_{II} + 1_{I}$ (note stickiness); *c*, monosomic 90–64–7 with $20_{II} + 1_{I}$ showing secondary associations of bivalents into groups of three or two bivalents, lower centre group of five bivalents and the univalent (arrowed); *d*, *L. perenne × F. rubra* hybrid with $12_{II} + 4_{I}$; note five ring bivalents.

Table 1 Chromosome pairing in some euploid and monosomic plants of *Festuca arundinacea*

Cross	2n	VI	V	IV	III	II	I	Chiasmata per cell	No. of cells analysed
Bn 273 × Bn 488									
Hybrid −1	42	—	—	—	—	21.00	—	32.83	30
−2	42	—	—	—	—	21.00	—	33.40	30
−3	42	—	—	—	—	20.90	0.20	30.97	30
−4	42	—	—	—	—	20.97	0.07	30.83	30
−5	42	—	—	—	—	20.97	0.07	31.80	30
Bn 488 × Bn 273									
Hybrid−2	42	—	—	—	—	21.00	—	31.00	20
−3	42	—	—	—	—	20.90	0.20	29.93	40
−6	42	—	—	—	—	20.97	0.07	30.90	30
Monosomic 90–64–7	41	0.04	0.03	0.27	0.20	18.80	1.31	32.93	96
−9	42	—	—	—	—	20.96	0.08	36.01	70
Other monosomics in tall fescue									
(i) 1–15–4	41	—	—	—	—	19.93	1.13	31.33	30
(ii) 17–78–2	41	—	—	—	—	19.84	1.31	33.80	45
(iii) 19–21–9	41	—	—	—	—	19.75	1.50	33.13	8

Bn 273, Tall fescue from Algeria.
Bn 488, Tall fescue from Israel.

study of meiosis in the tall fescue polyhaploid ($2n = 21$) in which a mean of 4.01_{II} and 12.96_{I} were observed with 7_{II} in nearly 18 % of the cells analysed[6]. These bivalents and a trivalent must have resulted from homoeologous pairing and is in accordance with the theory that the genetic system controlling bivalent pairing is ineffective in the hemizygous state. On the contrary, in wheat[1] and oats[3], the polyhaploids show very little chiasmate pairing because genetic control is effective in the hemizygous state. It also seems that the regulator in the hemizygous state in the monosomic 90–64–7 permits multivalent formation without markedly affecting chiasma frequency (Table 1).

It is interesting to note that there was a conspicuous tendency for secondary associations among bivalents in the monosomic 90–64–7. Chromosome spreading was more difficult because of stickiness (Fig. 1*b*), but even in well spread metaphase plates the secondary associations were clearly evident (Fig. 1*c*). Since secondary associations are now known to take place between bivalents of genetically and evolutionarily related chromosomes[7], it is reasonable to infer that the grouped bivalents have related chromosomes with synaptic potentiality and that they tend to be closely associated, presumably as a result of the relaxation of genetic control in this monosomic.

Further support for the existence of a regulatory system in tall fescue, which is not effective in its haploid complement, comes from the study of several other hybrids and amphiploids between tall fescue and diploid *Lolium* species. Thus, in the 28-chromosome hybrids between *L. multiflorum* ($2n = 14$) and *F. arundinacea* ($2n = 42$), Lewis[8] observed a maximum of 14_{II} in several cells, whereas the mean for three hybrids was 9.52_{II} per cell. Some of these bivalents, and associated trivalents and quadrivalents must have resulted from extensive homoeologous pairing resulting from the breakdown of the regulatory mechanism in the haploid complement of tall fescue. In the amphiploids ($2n = 56$), however, when its full complement was present, and hence the regulator in the double dose, normal chromosome pairing was largely restored. Up to 28_{II} (mean of three amphiploids $= 24.23_{II}$ per cell) were observed and the multivalent frquency was much lower compared to the 28-chromosome hybrids. Since the amount of differentiation between *Lolium* and *Festuca* chromosomes is very low and permits little preferential pairing[5], the synaptic behaviour of the *multiflorum–arundinacea* amphiploids can be explained only on the basis of genetic regulation.

As far as the author is aware, a hemizygous ineffective regulator of diploid-like pairing has not been recorded in any other polyploids of Gramineae or any other family. It may well be, however, of wide occurrence in the grass family. That other polyploid fescues have a similar regulator is borne out by the study of two intergeneric hybrids between *Lolium perenne* L. ($2n = 14$) and *Festuca rubra* L. ($2n = 6x = 42$). Whereas the

parents formed 7_{II} and 21_{II} respectively, the hybrids (Fig. 1*d*) showed a mean of $0.03_{IV} + 0.10_{III} + 11.57_{II} + 4.69_{I}$. Twenty-eight per cent of the cells had 13 or 14 bivalents, and the frequency of ring-bivalents was high (mean chiasmata $= 19.85$ per cell). It seems that there was extensive homoeologous pairing as a result of the hemizygous state of the regulator in the haploid *rubra* complement in these hybrids. In the hexaploid *rubra*, however, no homoeologous pairing is observed because of the fully effective regulator in double dose.

Similar results were obtained in another intergeneric cross between *L. multiflorum* ($2n = 14$) and *F. arundinacea* var. *letourneuxiana* ($2n = 70$) and its amphiploid[9]. Whereas meiosis in the parents is characterised by bivalent formation, the hybrids ($2n = 42$) formed a mean of 13.72_{II}, and several trivalents, quadrivalents, pentavalents and hexavalents. The amphiploid ($2n = 84$), however, showed almost regular meiosis forming predominantly bivalents (mean 38.27_{II} per cell, some cells with 42_{II}). These results of Malik and Thomas[9] and those of Chandrasekharan and Thomas[10] and of several earlier workers like Crowder[11] and Lewis[8] can be more meaningfully explained on the basis of the existence of a 'genetic regulator' in tall fescue and other polyploid fescues, which exercises its regulatory control on homoeologous pairing when it is present in the double dose but is haplo-insufficient.

The hemizygous ineffectiveness of the regulator is clearly of evolutionary significance in that, in the hybrids, it could have permitted the gene flow from one species to another, but subsequent chromosome doubling resulted in stable amphiploids in nature. This would at least partly account for the widespread introgression of characters between taxa.

The possible existence of such a regulator could have important implications for the cytogenetic relationships of the entire *Lolium–Festuca* complex and the strategy of the plant breeder in making use of this group. To avoid loss of the 'genetic regulator' even on one chromosome, more emphasis will have to be laid on incorporating the full complement of hexaploid fescues, for example, tall fescue and red fescue, in the production of agronomically superior, stable *Lolium–Festuca* amphiploids. The precise location of the regulator in tall fescue and other polyploids in the Festuceae could open up new avenues in forage breeding of the type being witnessed in wheat[12,13].

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Origin of extragonadal teratomas and endodermal sinus tumours

TERATOMAS are usually differentiated tumours, containing tissues derived from ectoderm, endoderm and mesoderm, and may arise in the gonads or elsewhere. Benign ovarian teratomas are parthenogenetic tumours derived from a single germ cell after the first division of meiosis, as studies of chromosome markers and enzyme variants have shown[1,2]. We have now found that extragonadal teratomas develop in a different manner. We have examined single gene products and chromosomes in extragonadal teratomas and report here our results. We include data on endodermal sinus tumours because of their similarity to teratomas[3,4].

phoretic phenotypes of the following enzymes were determined: adenylate kinase (AK), phosphogluconic dehydrogenase (PGD), phosphoglucomutase (PGM$_1$ and PGM$_3$), glutamic-pyruvic transaminase (GPT) and glucose-6-phosphate dehydrogenase (G-6-PD). HL-A typing was perfomed on Terasaki plates[5] after mild trypsinisation.

The results showed that in four cases normal host tissue was heterozygous at seven loci (Table 1). In all seven situations when the host was heterozygous the tumour was also heterozygous.

Chromosome studies showed that the teratoma of case 3, the patient with Down's syndrome, had trisomy 21 (47,XX,+21) (Fig. 1). If this tumour had been derived from a germinal cell after meiosis, two or four doses of chromosome 21 would have been expected. But there were three doses, as anticipated with normal mitosis. The 46,XY karyotype in the endodermal sinus tumour from a male (case 5) is likewise consistent with mitosis rather than meiosis at the time of inception of the tumour. A 46,XY chromosome constitution has also been observed[6] in a benign sacrococcygeal teratoma.

Thus all our results suggest that these tumours develop from a mitotic cell. The cell of origin could be either a somatic cell or a
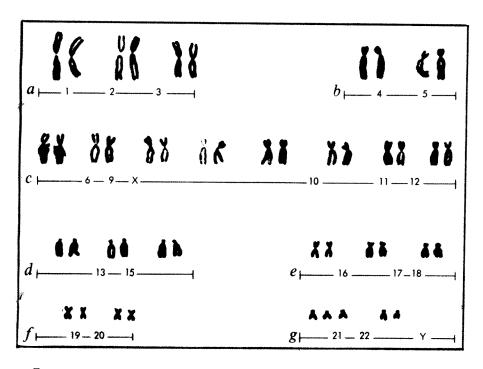
Fig. 1 Karyotype analysis of cultured cells from a retroperitoneal teratoma in a person with Down's syndrome. The normal host cells and those of the teratoma are 47,XX,+21.

Extragonadal tumours and normal tissues were obtained at surgery from five patients (Table 1). One sacrococcygeal teratoma and two retroperitoneal teratomas were benign, while the two endodermal sinus tumours were malignant. Four of the patients were female. One (case 3) had Down's syndrome.

Tissues were grown in monolayer cultures and the electro-

misplaced germ cell which failed to undergo meiosis and had proceeded directly into mitosis.

G-6-PD data may shed light on the question of whether the tumours have a unicellular or multicellular origin. This enzyme is X-linked[7], and in normal female cells one of the two alleles (*A* or *B*) undergoes random, irreversible inactivation[8,9] early in

Table 1 Phenotype of extragonadal teratomas and endodermal sinus tumours

Case	Age	Sex	Tissue	Site	AK	PGD	PGM$_1$	PGM$_3$	GPT	G-6-PD	HL-A 1st series	HL-A 2nd series	Karyotype
1	Newborn	F	Normal		1	A	1	2–1	1	AB	2,3	?	—
			Teratoma	Sacrococcygeal	—	—	—	2–1	1	AB	2,3	?	—
2	2 months	F	Normal		1	A	1	1	2–1	—	—	—	—
			Teratoma	Retroperitoneal	—	—	—	—	2–1	—	—	—	—
3	7 months	F	Normal		1	A	2	2	2–1	—	—	—	47,XX,21
			Teratoma	Retroperitoneal	—	—	—	—	2–1	—	—	—	47,XX,21
4	26 months	F	Normal		1	A	2–1	1		—	2,9	7,12	—
			Endodermal sinus tumour	Sacrococcygeal	—	—	2–1	—	—	—	2,9	7,12	—
5	4 yr	M	Normal		1	A	1	—	—	—	—	—	46,XY
			Endodermal sinus tumour	Sacrococcygeal	—	—	—	—	—	—	—	—	46,XY
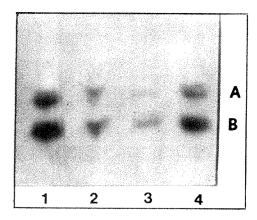
Fig. 2 Starch gel electrophoresis of G-6-PD isozymes performed on cell cultures. Normal host cells (1,2) and cells from a sacrococcygeal teratoma (3,4) are heterozygous with an AB phenotype.

embryonic development[10]. G-6-PD heterozygotes with an AB phenotype have two populations of somatic cells, one with the A and the other with the B phenotype. Some tumours such as leiomyomas of the uterus manifest only one G-6-PD phenotype (A or B), suggesting their origin from a single cell[11]. Tumours with a heterozygous G-6-PD phenotype[12,13] are thought to have originated from two or more cells.

The G-6-PD phenotype of the host in case 1 was AB, and so was her sacrococcygeal teratoma (Fig. 2). This teratoma could have originated from several cells, at least one of which was A and the other B. This presupposes that the tumour arose after X-inactivation. The times of X-inactivation and tumour inception in man are, however, too imprecise to know which came first: X-inactivation or the embryonal tumour. Another possibility therefore is that the tumour arose from a single cell before X-inactivation and that by chance in some cells the *A* allele was inactivated, while in others it was the *B* allele. A third possibility—that the teratoma arose from a cell with both G-6-PD alleles active—can be excluded, since no AB hybrid band was observed[14,15].

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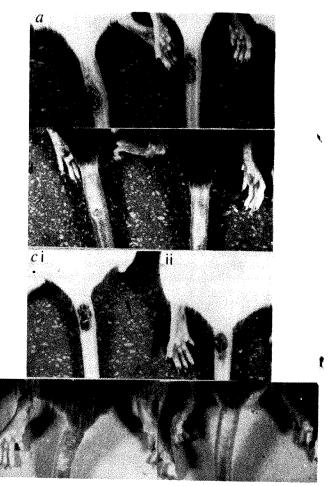
Reaction of mouse strains to skin test for ectromelia using an allied virus as inoculum

Ectromelia, or 'mouse pox', is a highly infectious virus disease of mice which can be present as a subclinical enzootic or a clinically apparent epizootic condition. As a result of importation of infected mice, an outbreak of this disease occurred in research establishments in the London area during late 1973 and the early part of 1974. These mice showed immunological evidence of infection without overt signs of disease, and those in contact were affected by the acute form of the infection. Detection of the infection is usually effected by one of three methods: inoculation of suspect material on to the choriollantoic membrane of 10-d-old chick embryos, in which typical pocks lesions are produced 3 d later if virus is present; serologically, using haemagglutination test[1]; using the tail scarification test with vaccinia virus, a closely related pox virus, as inoculum.

During the outbreak, screening of large numbers of mice by the Laboratory Animals Centre (LAC) was carried out using the tail scarification method as the principal test. Marked variations in response to inoculation were observed between the different inbred strains screened. Briody[1] gave 1.0×10^4 pock-forming units (PFU) of IDH-E strain of vaccinia as a vaccination dose and noted that reaction to the inoculation varies with the strain at this level of infection, but that at 1.0×10^6 a typical lesion should result.

We have compared the effect of inoculation on a range of specific-pathogen-free (SPF) inbred and non-inbred mice

Fig. 1 *a*, AKR strain reaction to Lister vaccinia. *b*, NZB strain reaction to Lister vaccinia. *c*, A strain reaction to (i) Lister, (ii) LAC vaccinia. *d*, B10.D2 strain reaction to (i) Lister, (ii) LAC vaccinia.

Table 1 An assessment of the reaction of inbred strains to two vaccinia vaccines

Strain	Lister vaccine	LAC vaccine	Strain	Lister vaccine	LAC vaccine	Strain	Lister vaccine	LAC vaccine	Strain	Lister vaccine	LAC vaccine
A	++++	++++	C57L	+++	++	CBA/Ca	+++	+++	CBA/Y*	+++	ND
AKR	++++	+	LACG	++++	+	NZB	+	0	B10 Y*	+++	ND
A2G	++++	+	C57BL	+++	+++	+/nu	++/+	0/+	HTT*	+++	ND
NMRI	+++	++	C57BR	+++	+	129	+++	0	AKM*	+++	ND
BALB/c	+++	++/+	C57BL/10ScSn	+++	+++	NIH	+++	ND	4R×5R(F₁)*	+++	ND
LACA	++++	+++++	B10 A	+++	0/+	CBAT6*	+++	ND	4R*	++	ND
ICFW	+++	+	B10 BR	++	+	B10A×4R*	+++/+	ND	5R*	+++/++	ND
NZW	+++	++	B10 D2	+++/+	0	CSI*	+++/+	ND	—	—	—
CE	+++	5×0/+	B10 LP-a	+++	+	CRH*	+++/+	ND	—	—	—
DBA/1	++++	0/+	C3H/He-mg	++++	+	A/Jax*	+++	ND	—	—	—
DBA/2	+++	+++	C3H/He	+++	+	PY*	+++	ND	—	—	—

ND, not done, 0, negative, +, positive Grades of reaction represented by number of plus signs
Mixed reaction represented by subscript plus   *Groups of three mice only

known to be free of ectromelia A total of 24 different strains maintained within the SPF unit of the LAC were tested for their response to the Lister strain of vaccinia and also to a weak strain of the virus donated to the LAC 4–5 yr previously As the strain of this virus is not definitely known, it will be referred to in this paper as the 'LAC' strain Observations were also made on other strains of mice sent for screening on which only the Lister vaccine was used

The virus antigen used for screening was freeze-dried small-pox vaccine (Lister Institute) in 25 dose ampoules for human use, and had approximately $1 0 \times 10^9$ PFU ml^{-1} The weak LAC strain originating from a human vesicle was passaged four times through eggs and assessed as being less than $1 0 \times 10^2$ PFU ml^{-1} Groups of 6 SPF mice between 6 and 8 weeks of age from each of the following strains were used for testing both Lister and LAC vaccine A, A₂G, AKR, B10 A, B10 D2, B10 LP-a, BALB/c, C3H/He, C3H/He-mg, C57BL/10ScSn, C57BL, C57BR, C57L, CBA/Ca, CE, DBA/1, DBA/2, ICFW, LACA, LACG, NMRI, +/nu, NZB Other strains, using the Lister vaccine only, were 129, AKM, B10 A(4R), B10 Y, CBA/H-T6, CBA/Y, CRH, CSI, HTT, NIH, B105R, PY All groups of mice, whether from the SPF unit or sent from outside for screening, were placed in PVC boxes fitted with a filter lid made from sterilised FG50 glass fibre filter material After inoculation, the groups were maintained in similar filter containers until tested

A small area of the dorsal surface at the base of the tail was carefully scraped with a sterile scalpel until the upper epidermal layers were removed and blood was drawn A microdrop of the virus antigen was placed on the scarified area, and the test read 8–10 d later In a positive reactor, the site of inoculation was usually swollen with either a raised pinkish cream blister or had a reddish focal area in the centre of the oedematous tissue In non-reactors the site of scarification had completely healed and returned to normal

Results obtained from the majority of mice inoculated with Lister vaccinia vaccine were good, and those of the LAC vaccine variable (Table 1) A poor response to the Lister strain was noted in only one of the coloured strains, the NZB Fig 1b, a mixed response in five groups, the B10 D2 and +/nu, B10 A(4R), CSI and CRH All albino strains responded very well to the Lister vaccine with very little variation and Fig 1a shows a typical example of a good reaction in AKR strain The coloured strains varied in response slightly but all were recognisably positive (Table 1) Strains A, DBA/2 and C57BL/10ScSn reacted well to the LAC vaccine, as did LACA, C57BL, CBA/Ca The NMRI and ICFW strains, on the other hand, showed a reduced reaction, the C3H/He-mg produced a very weak response and the B10 D2, NZB and the 129 strains were all negative (Fig 1c and d) Variation in response to the Lister vaccine by individuals within a strain was on the whole very limited Some B10 D2 and +/nu did not give a marked response, but all were clearly positive In all other strains the six mice in each group tended to show a uniform reaction

Despite the very low PFU of the LAC vaccine, reactions equivalent to the Lister vaccine occurred in six of the strains tested—A, LACA, DBA/2, C57BL, C57BL/10ScSn and CBA/Ca Three strains showed marked variation—the +/nu, DBA/1 and B10A—which all had mixed positive and negative reactions Three strains were clear negatives—B10 D2, NZB and 129 All other strains, although eliciting a weaker reaction than with the Lister strain, gave clear positive reactions Thirteen other strains were tested with the Lister vaccine Fewer mice were available for testing, but each gave very good positive reactions (Table 1)

The tail scarification method seems to be a very useful method of screening, provided it is carefully carried out It does not seem likely that false negatives would be found using the Lister vaccine with its high PFU Of 37 strains tested all produced a recognisably positive result, and it is fairly easy to read even in poor reactors, as the blisters, however small, are unmistakable It is important, however, that wherever possible the same operator should perform the scarification and application of the antigen, to ensure uniformity of technique The importance of uniformity in technique was indicated by the tests run on the CE strain As a result of a change of technician, irregular results were obtained from both vaccinated groups The test was rerun and uniform results recorded from both groups Also, suitable controls are necessary when testing inbred strains Where possible, SPF mice of the same strain should be used as controls whenever suspect colonies are to be screened

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Mitigation of virus-induced foetal growth retardation in mice by dietary casein hydrolysate

EXPERIMENTAL infection of pregnant mice with Coxsackievirus B3 induces retarded foetal growth and development of the plasma proteins[1,2] Subsequent work in mice indicated that the clinically mild illness induced by the virus in the mother may be largely responsible for the impaired foetal development, even though a direct effect of the virus in the foetus could not be ruled

Table 1 Influence of dietary casein hydrolysate supplement on foetal development in mice infected with Coxsackievirus B3

Days of pregnancy when casein hydrolysate is given in diet	Treatment	No of animals	Maternal weight (g)	Total no of live foetuses	Total no of resorptions	Foetal weight (g)	Foetal plasma Alb/AFP
1–18	Live Coxsackievirus B3 +10% casein hydrolysate	10	40 200±1 520	75	13	1 143*	0 761†
	Live Coxsackievirus B3	16	31 987±1 057	52	39	0 806	0 526
	Heat-killed Coxsackievirus B3	8	45 926±1 823	80	0	1 248	0 807
10–18	Live Coxsackievirus B3 +10% casein hydrolysate	15	38 251±1 448	109	13	1 194	0 792
	Live Coxsackievirus B3	11	36 742±1 700	58	21	0 838	0 565
	Heat-killed Coxsackievirus B3	9	47 990±1 600	89	3	1 400	0 946

* Standard error foetal weight ±0 172
† Standard error Alb/AFP ratio ±0 103

out[3] The infection resulted in maternal pancreatitis within 2 d, with the animals being incapable of digesting adequate amounts of important dietary constituents, notably proteins, to maintain normal foetal growth[4], in spite of a higher intake of food by the infected mothers We concluded that the pancreatic lesions resulted in infected animals being deprived of essential dietary proteins, and therefore determined whether the effects of the pathogen could be mitigated or perhaps overcome by the administration of a readily assimilable diet

Pregnant mice (20–25 g on day 1 of pregnancy) were fed a powdered diet for breeding rodents supplemented with 10% w/w casein hydrolysate (Sigma, St Louis) On day 8 of pregnancy 0 3 ml of a suspension of Coxsackievirus B3 containing $10^{5.85}$ ml^{-1} of $TCID_{50}$ was given intramuscularly A second group of mice, which received no dietary supplement, was also injected with the same virus suspension

Table 1 shows that the foetuses from the supplemented group at day 18 of gestation, when the experiment was terminated, did not differ significantly in weight from the controls given heat-killed virus suspension, whereas those which had no supplement were significantly lower in weight ($P < 0.01$)

In a second series of experiments dietary supplement was not given until 2 d after the virus suspension was administered The group of mice which had received the supplement was significantly heavier (Table 1) than those which were given live virus alone ($P < 0.01$), but similar in weight to the controls which received heat-killed virus

The plasma protein pattern from each group of foetuses was examined by two-dimensional immunoelectrophoresis Groups which received virus alone had a low albumin/α_1-foetoprotein ratio (Alb/AFP), which has been shown previously to be associated with retarded foetal development[1,2] The Alb/AFP ratio of foetuses from virus-infected mice receiving a diet supplemented with protein hydrolysate was only slightly lower than that of the control group given heat-killed virus (difference not statistically significant) The degree of mitigation was independent of the time when feeding of the dietary supplement began

These results indicate that supplementation of the diet of pregnant mice infected with Coxsackievirus B3, by the addition of suitable quantitites of simple peptides and amino acids in the form of protein hydrolysate, can mitigate the effects of the virus on foetal growth retardation It seems that the dietary supplementation compensates for foetal protein deficiency arising as a consequence of the virus-induced destruction of maternal pancreatic exocrine tissue Immunoelectrophoretic studies of foetal plasma proteins show that this mitigation is not merely limited to increasing the weight of the foetus but also extends to annulling the effects of the virus on the development of the normal plasma protein patterns

Although the results of these studies apply to Coxsackievirus B3 infection, it seems possible that they may also apply to other infections which adversely affect foetal growth indirectly by their pathological effects on maternal organs necessary for optimal foetal development

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Mycobacterium microti may protect itself from intracellular destruction by releasing cyclic AMP into phagosomes

PATHOGENIC mycobacteria are able to survive and multiply within macrophages How they do this is not known, but recent quantitative electron microscope surveys of intracellular events in macrophages infected *in vitro* with either *Mycobacterium tuberculosis* or *M microti* revealed a lack of discharge of lysosomes into phagosomes containing live bacilli, whereas dead bacilli were associated with discharged lysosomal contents within phagolysosomes[1,2] Possibly the living bacteria produce a factor which inhibits fusion between phagosomal and lysosomal membranes and thereby prevent the discharge of putatively bactericidal lysosomal contents into the bacterial environment Since high intracellular levels of cyclic adenosine 3',5'-monophosphate (cyclic AMP) mediate pharmacological inhibition of lysosomal discharge in leukocytes[3,4] we reasoned that increased amounts of this nucleotide may occur in macrophages infected with living mycobacteria but not in those infected with dead ones We report here experiments with *M microti* showing that this is so and suggesting a bacterial origin for the additititional cyclic AMP

Marked elevation of the cyclic AMP content was observed only in monolayers ingesting live bacilli (Fig 1) Minor disparities in the rates of association of the live and heat-killed bacteria with the macrophages as detected by light microscopy could not account for the difference The increase in cyclic AMP correlated ($P < 0.01$) with the number of cell-associated live bacilli (Fig 2) and was remarkable since in other studies human polymorphonuclear leukocytes showed no change in cyclic AMP content during phagocytosis of latex spheres[9,10] or zymosan[11] Furthermore, macrophages ingesting either bacilli killed by ultraviolet irradiation, latex spheres, colloidal gold or *M lepraemurium* showed little or no increase in cyclic
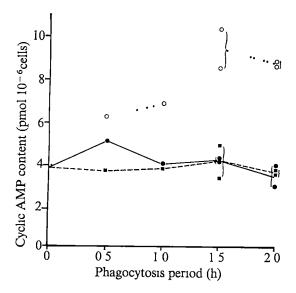
Fig 1 Macrophage cyclic AMP content during phagocytosis Macrophage monolayers were obtained from CFLP mice (Anglia Alconbury, UK) and 6–13 d later exposed to bacteria and other ingestible particles as described previously[1] except that the cells were maintained in plastic Petri dishes (diameter 5 cm) with about 1 0 × 10⁶ cells per dish M microti was grown in a chemostat in an asparagine–casamino acid medium (Dubos Broth Base, × 4 concentration, Difco) at a dilution rate of $D = 0 016$ h⁻¹ and about 8 × 10⁸ organisms ml⁻¹ Samples were centrifuged, resuspended in infection medium (2 5% horse serum in Hanks' balanced salt solution (BSS)) to yield 1–8 × 10⁸ bacilli ml⁻¹, essentially free of clumps and 100% viable BSS-rinsed monolayers were overlaid with the suspension at a bacteria–cell ratio of 300–2,200 1 for 2 h at 37 °C Monolayers of 8 4 × 10⁵ macrophages were incubated with 4 ml infection medium either alone (■) or containing 2 0 × 10⁸ live (○) or heat-killed (●) M microti ml⁻¹ At 0 5 h intervals monolayers were chilled on ice, rinsed twice with ice-cold BSS and the majority of the cells scraped free into 2 ml BSS After adding 1 ml 12% (v/v) perchloric acid they were briefly ultrasonicated and duplicate aliquots assayed for DNA (ref 5) and cyclic AMP Cyclic AMP was assayed using a competitive protein binding method[6] after initially purifying neutralised aliquots on AG50W–×8 H⁺ resin by washing with 1 mM potassium phosphate buffer, pH 7 0, and eluting with water[7] The number of macrophages per monolayer was calculated from the DNA recovered (28 6 μg = 10⁶ cells) Since bacterial DNA contributed significantly to the total DNA in monolayers exposed to bacteria, the mean of the DNA contents of the resting (infection medium alone) monolayers was used throughout for calculating the cyclic AMP–cell ratios Typically, the coefficient of variation of the resting cell DNA values was about 12% The rates of phagocytosis of living and of dead bacteria were similar, as estimated by light microscopy of the residual monolayer cells stained using Kinyoun's modification of the Ziehl–Neelsen stain[8] Random fields were examined until a total of 200 cells had been counted, the numbers of cells containing 0, 1–10, 11–20, 21–50 or > 50 bacilli were recorded and the mean number of bacilli per cell calculated assuming 0, 5, 15, 35 and 60 per cell in each respective score group

AMP (Table 1) The lack of response with M lepraemurium, in spite of an average of up to 12 cell-associated bacilli per cell, is consistent with the lysosome–phagosome fusion observed with this organism[2] its success as an intracellular parasite may result from a special lipid coat protecting against inimical lysosome contents[12]

That the elevation of cyclic AMP in monolayers exposed to live M microti was not caused by the cellular biochemical events of phagocytosis was suggested by experiments in which monolayers were thoroughly rinsed after a phagocytosis period of 2 h and incubated for a further 2 or 4 d in maintenance medium[1] before assay Increased levels of cyclic AMP were still apparent (Table 1) In this instance the lysosomes were labelled with colloidal gold before phagocytosis of mycobacteria so that lysosome–phagosome fusion could be assessed by electron microscopy[1] of monolayers treated identically to

those assayed for cyclic AMP Phagolysosome formation, as revealed by electron-dense gold particles in the same vesicles as bacilli, was detected only in cells exposed to dead organisms

Although a contribution from macrophage metabolism has not been discounted, the additional cyclic AMP in M microti infected cells could have originated from the bacteria as shown by measurement of their cyclic AMP content and generating capacity Typically, 2×10⁷ bacteria from the chemostat contained 0 24 pmol, this was not changed significantly after 2 h at 37 °C in infection medium but it was lost after heat-killing Thus, if their cyclic AMP content was unaltered during the infection process, 20 live bacteria per cell would have contributed 0 24 pmol to 10⁶ macrophages This is inadequate to account for the 4 pmol increase observed (Fig 2) Allowance must be made for possible underestimation of bacterial numbers by light microscopy with heavily infected cells and for possible increase in the rate of bacterial cyclic AMP synthesis within phagosomes Preliminary studies revealed that the environment of the bacillus can affect cyclic AMP synthesis sufficiently to remove the discrepancy 2 × 10⁷ organisms produced 4 pmol cyclic AMP within 1 5 h at 37 °C in phosphate buffered saline (Dulbecco A, Oxoid, London)

M microti inefficiently retained the synthesised nucleotide, in the chemostat 80% was extracellular and in phosphate buffer 88% was released within 3 5 h The bacillus clearly possesses potential for liberating cyclic AMP into phagosomes in amounts which may inhibit lysosomal fusion Mammalian cells are relatively impermeable to exogenous cyclic AMP (ref 13) If the 3–7 pmol cyclic AMP increment detected 4 d after infection (Table 1) was produced by the bacilli residing in phagosomes of 1 5 × 10⁻¹⁵ (the approximate bacterial volume) impermeable to cyclic AMP an intraphagosomal concentration of about 7 × 10⁻⁵ M could be inferred from the number of bacteria present This is similar to the 10⁻⁴ M extracellular cyclic AMP which inhibited macrophage lysosome fusion[3]

Other successful intracellular pathogens which are able to prevent lysosome–phagosome fusion are Toxoplasma gondii[14], chlamydia[15] and perhaps Neisseria gonorrhoeae[16] Measurements of cyclic AMP levels in cells infected with these agents

Fig 2 Dependence of macrophage cyclic AMP content on degree of infection Data were pooled from five similar independent experiments in which levels of cyclic AMP were determined in macrophages which had been either resting or ingesting live or heat-killed M microti for up to 2 h Symbols and experimental details as in Fig 1 except that the difference in cyclic AMP content between ingesting and resting cells is plotted and the number of macrophages per monolayer and the dose of bacteria varied between experiments Regression of y on x was fitted by the least squares method
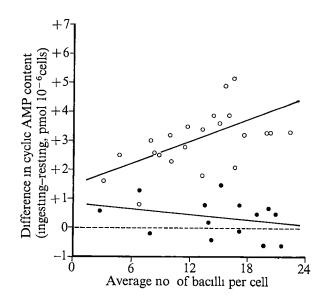
Table 1 Cyclic AMP content of macrophage monolayers exposed to a range of ingestible particles

Treatment	Experiment 1 after contact with particles for		Experiment 2 after contact with particles for		Experiment 3 exposed to particles* and then maintained for a further	
	1 h	2 h	1 h	2 h	2 d	4 d
Control	1 7	1 7	2 4	3 0	3 7	6 1
	1 4	2 1	2 7	2 8	4 8	5 7
Live *M microti*†	4 9	5 0	5 0	6 9	7 4	10 8
	4 0	4 5	5 7	6 7	7 1	9 4
	—	—	5 8	6 3	8 0	12 6
Heat-killed	—	—	—	—	3 9	5 2
M microti†	—	—	—	—	3 6	4 3
	—	—	—	—	—	6 2
Lethally ultraviolet	2 8	2 6	—	—	—	—
irradiated *M microti*†	2 2	2 2	—	—	—	—
Latex spheres‡	2 3	1 5	—	—	—	—
	1 7	2 3	—	—	—	—
M lepraemurium§	2 9	2 3	—	—	—	—
	2 4	2 6	—	—	—	—
Colloidal gold¶	—	—	3 1	3 0	—	—
	—	—	3 2	2 1	—	—
	—	—	2 7	2 1	—	—

Measurements on individual monolayers are shown

*All monolayers in Experiment 3 were treated first with 2 5 ml of colloidal gold (particles of up to 20 nm diameter, Radiochemical Centre, Amersham) which had been diluted to 500 µg ml^{-1} in infection medium

†The suspension of *M microti* used presented a bacteria–cell ratio of 1,200, 2,200 and 500 1 in experiments 1, 2 and 3, respectively

‡A suspension of microspheres with a mean diameter of 500 nm (Polaron Equipment, Watford, UK) was diluted in infection medium and used at a particle–cell ratio of 1,900 1

§*M lepraemurium* (Dr R J W Rees) was a partially purified suspension obtained from mouse spleen and containing 6 6×10^{11} bacilli ml^{-1} in 0 1 % (w/v) bovine serum albumin saline It was diluted with infection medium and used at a bacteria–cell ratio of 1 2×10^5 1

¶Obtained and used as described above*

Other experimental details were as described in Fig 1 and in the text

might reveal the nucleotide as a common mediator of the infection-induced paralysis of intracellular digestion

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Spontaneous progressive multifocal leukoencephalopathy (PML) in macaques

PROGRESSIVE multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system (CNS) which has so far been recognised only in man[1,2] Although it is an infrequent cause of clinical neurological disorder[3], it is of particular interest because of its frequent association

with diseases in which immunological competence is impaired[4-7], and because it is the only chronic demyelinating disease of man in which a virus (papovavirus) has been consistently demonstrated[4,5,8,9] So far, efforts to transmit the disease to non-human primates have failed[8] This report of a spontaneous disease in macaques with striking similarities to PML seems to be the first recorded instance of such a condition in animals

While reviewing the histology of approximately 400 monkey brains collected at autopsy, we found eight macaques (seven *Macaca mulatta* and one *M speciosa*) with lesions similar to those described for PML Electron microscopic examination of formalin-fixed brain tissue revealed papova-like virions in six of these monkeys

A precise age (Table 1) was known for the two animals born in captivity The remaining six were captured in the wild and had been at this centre 23–64 months, all were adults estimated to be over 8 yr old The disparity in sex—seven females and one male—is a reflection of sex distribution of monkeys of this age in the colony All monkeys received isoniazid in their diet while at the centre as prophylaxis for tuberculosis (227 mg isoniazid and 11 37 mg pyridoxine per pound of food)

Macroscopic lesions consisting of soft translucent foci were recognised when slices of fixed brain tissue were examined closely These foci were usually 1–2 mm wide, and were randomly located, mainly in the subcortical white matter (Fig 1), hypothalamus, medulla oblongata and adjacent to the cerebral aqueduct A firm nodule, 1 cm in diameter, was present in the basilar meninges and compressed the adjacent brain in case 7 In case 6, the left optic nerve was encased by a layer of fibrous tissue which extended to the eyeball

Histological foci of demyelination were present in all brains Larger demyelinated areas seemed to result from confluence of smaller foci Most demyelinated foci encompassed or were immediately adjacent to blood vessels

Microglia and large astrocytes (gemastete glia) were present in and adjacent to foci of demyelination Some nuclei of the oligodendrocytes in these areas were enlarged Large polygonal cells, judged to be of astrocytic origin, with eosinophilic fibrillar cytoplasm and large vesicular nuclei, were present among the cellular accumulations (Fig 2) These cells often contained
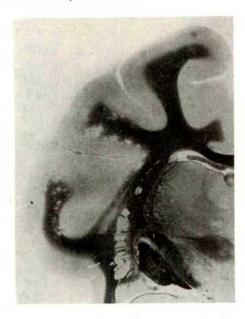
Fig. 1 Foci of demyelination typical of the changes present in monkeys with PML. (Luxol Fast Blue × 2.3).

multiple nuclei and on rare occasions mitotic figures. Similar focal cellular accumulations were present in the grey matter.

Basophilic and eosinophilic intranuclear inclusion bodies were present in oligodendroglia and astrocytes of all eight monkeys. Cells with inclusions were in or adjacent to lesions in the grey and white matter but varied considerably in frequency from case to case. Eosinophilic inclusions were usually separated from a ring of marginated chromatin whereas the basophilic inclusions filled the nucleus (Fig. 2).

Perivascular cuffs consisting of lymphocytes, monocytes and rarely plasma cells were present in all brains. In three cases the cuffs were prominent but in the other five they were minimal to moderate in extent.

The spinal cords of seven monkeys were examined. Lesions similar to those in the brain were present in four. The optic nerve and retina were similarly affected in two out of five monkeys.

The nodule in the basilar meninges of case 7 and the fibrous tissue surrounding the left optic nerve of case 6 were interpreted as unusual fibroproliferative lesions. A marked reactive gliosis was present in the neuropil adjacent to these meningeal lesions. A similar nodule approximately 0.5 mm in diameter was present in the left frontal lobe of case 5.

Electron microscopic examination was carried out on formalin-fixed brain tissue which had been processed in the usual fashion and embedded in Epon, or, on paraffin sections stained with haematoxylin and eosin which had been re-embedded in Epon[10]. Particles which were round, without envelopes and approximately 30 nm in diameter were found in

cells with inclusion bodies. Some particles were uniformly dense while others consisted of a dense peripheral ring (capsid) surrounding a lucent core. The particles were usually numerous and randomly distributed throughout the nucleus (Fig. 3). Occasionally large or small aggregates formed a lattice or crystalloid arrangement. Viral particles were rarely found in the cytoplasm and no filamentous forms have been observed. In our experience in monkeys, these particles are unique to the cases described here.

Most human cases of PML have occurred in elderly patients with lymphomas or chronic inflammatory processes[1,2]. Others have been associated with immunosuppressive therapy[4,11].

The disease is characterised clinically by progressive neurological abnormalities lasting 3–12 months[3]. In rare cases patients have shown no neurological signs[8] or had no other concomitant disease processes[12-14].

The histological changes are characterised by focal demyelination[1,2]. Large oligodendrocytes and astrocytes, some of the latter having been described as giant and bizarre, are

Fig. 2 Photomicrograph from the periphery of a focus of demyelination demonstrating the typical cellular reaction. A giant cell and a basophilic nuclear inclusion body are present. (Haematoxylin and eosin × 560).

found in and adjacent to the demyelinated foci. Intranuclear inclusion bodies can usually be found in the oligoglia and less frequently in astroglia. An inflammatory cellular reaction is variable, being absent or modest in most cases but prominent in others.

While immunological competence was not determined in the monkeys reported here, observations in other monkeys having

Table 1 Summary of spontaneous progressive multifocal leukoencephalopathy in eight macaques

Case no.	Species	Sex	Age	Neurological signs	Concomitant disease(s)	Severity of CNS lesions	Papova-like virions in glia
1	*M. mulatta*	Male	Adult		Chronic haemolytic anaemia, mild giant cell pneumonia	+	+
2	*M. mulatta*	Female	Adult	Anisocoria, progressive rear-leg paralysis for 12 d	Moderate giant cell pneumonia	+++	+
3	*M. speciosa*	Female	Adult		Chronic colitis (shigellosis), mild giant cell pneumonia	+++	+
4	*M. mulatta*	Female	Adult				+
5	*M. mulatta*	Female	Adult		Lymphoma, Avian TB, chronic anaemia	++	
6	*M. mulatta*	Female	Adult		Lymphoma, intestinal amyloidosis	++	+
7	*M. mulatta*	Female	7 yr	Lethargic	Lymphoma, Avian TB	++	+
8	*M. mulatta*	Female	6 yr	Ataxia for 5 d	Chronic colitis (shigellosis), chronic vegetative endocarditis	++	
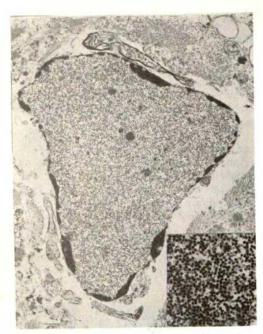
Fig. 3 Electron micrograph demonstrating papova-like virions in the nucleus of a glial cell. ($\times 3{,}780$, inset $\times 17{,}955$).

spontaneous lymphoma or avian tuberculosis suggest impairment occurs (T. G. Terrell and C. A. Holmberg, personal communication). Isoniazid had been included daily in the diet of these monkeys and it has been suggested that this drug may impair immunological competence also[15,16].

Jungherr *et al.*[17] examined the CNS of macaques experimentally infected with a papovavirus (SV40), and described non-suppurative inflammation. Hypertrophied, or 'pseudo-giant', ependymal cells were observed in some cases but no mention was made of inclusion bodies, or demyelination. All animals were killed on days 17–21 after inoculation.

We believe that the CNS disease described here in macaques has more similarities than differences to PML in man, and is a manifestation of infection by a papovavirus. Further search and evaluation of the factors causing the disease in monkeys may give insight into PML in man and other chronic viral infections of the CNS.

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Helper effect of normal and irradiated thymus cells on transferred immunoglobulin production

The cellular transfer of immunoglobulin (Ig) production in mice by various lymphoid cell preparations has been demonstrated previously by the use of allotype congenic mouse strains[1,2]. Lymphoid cell suspensions from one mouse line are injected intravenously into histocompatible but allotype-different, partner-strain mice without deliberate immunisation, and the resulting Ig production ascertained by donor allotype analysis of the sera taken at various times. We have used this transfer system to determine whether anti-μ pretreatment of lymphoid cells results in suppression of subsequent IgG production[2], and in studies on the radiosensitivity of the B cell series (R.E.A., and N.L.W., unpublished). In analysing the results of Ig transfer in such experiments, it has become evident that the possible role of T cells in either augmenting or suppressing the Ig transfer by B cells must be determined, and here we present evidence that such T cell involvement can be demonstrated to varying degrees.

Immunoglobulin transfer between three pairs of allotype congenic strains was studied. BALB/c (Ig^a allotype) and C57BL/6 (Ig^b) mice were used as recipients for spleen cell transfers from the respective donor strains BABB-14 (Ig^b allotype), (Herzenberg), BALB/c.Ig^b N.12 (Ig^b allotype), and C57BL/6.Ig^c (Ig^c allotype) (both Hall Institute). All donor mice were homozygous at Ig loci and were derived by inbreeding after 14, 12 and 6 backcross generations, respectively. Single cell suspensions of donor spleens were prepared from 6–10 week old mice by teasing spleens through stainless steel sieves into 10% foetal calf serum in MEM. All cell transfers were made intravenously to groups of five to eight recipient mice 6–10 weeks of age, that had received 400 rad whole-body irradiation within the previous 24 h. Recipient mice were bled at various intervals and the levels of donor type IgG$_2$ allotype quantitated by radioimmunoassay[3]. All results are expressed as the amount of donor type IgG as a percentage of a standard

Fig. 1 Effect of added thymus cells on Ig transfer by allotype congenic spleen cells. Donor spleen cells—4×10^6 C57BL/6.Ig^c. All thymus cells added at 1:1 ratio. ●, Non-irradiated 7 week old thymus cells; ○, 1,000 rad 7 week thymus cells; △, Non-irradiated 7 month thymus cells. Amount of donor type (Ig^c) Ig was assayed 1, 2 and 4 weeks after transfer and the relative amount of Ig in recipients of added thymus cells expressed relative to the amount with spleen transfer alone. Vertical bars and points show mean $\pm$ s.e.
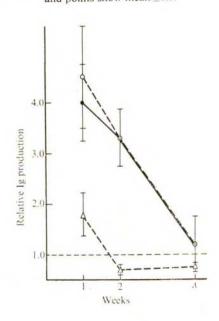
Table 1 Effect of T-cell depletion on Ig transfer

Experiment no	Donor	Transfer conditions Recipient	No of spleen cells	Assay time (d)	Percentage donor Ig (mean±s e)
1	BABB	BALB/c	7×10^6	7	1 7±0 3
	Anti-θ BABB	BALB/c	7×10^6	7	0 9±0 1
	BABB	BALB/c	7×10^6	21	5 4±1 2
	Anti-θ BABB	BALB/c	7×10^6	21	4 6±1 0
2	BABB	BALB/c	8×10^6	7	1 2±0 2
	Anti-θ BABB	BALB/c	8×10^6	7	0 9±0 1
3	C57BL Ig^e	C57BL	4×10^6	28	36 8±6 7
	Anti-θ C57Bl Ig^e	C57BL	4×10^6	28	48 6±7 5
4	CBA nu/nu	700 rad C57BL	10×10^6	7	3 1±0 3
5	Anti-θ BALB/c Ig^b N12	BALB/c	5×10^6	14	23 2±2 0*
	Anti-θ BALB/c Ig^b N12	BALB/c nu/nu	5×10^6	14	15 6±4 1†

*All mice (21 out of 21) showed donor type Ig levels in the range 10–40%
†Mean donor level for all mice (17) includes ten mice with ratios in the range 9–40% and a group of seven mice all having <2% donor Ig
The mean value for the ten mice showing >9% donor Ig was 25 8±4 7%, that is, similar to that in the 21 out of 21 intact recipients

adult serum pool of donor strain type In experiments on the addition of thymus cells, cell suspensions were similarly prepared from thymuses of mice of various ages that were syngeneic to the recipient strain

As most of the previous cell transfer experiments have involved whole spleen cell preparations (that is, T and B cells), transferred into sublethally irradiated but otherwise intact recipients (that is, the recipient also has T cells), we first determined the effect of eliminating T cells from the donor inoculum with anti-θ treatment In three such experiments (Table 1, experiments 1–3), anti-θ treated spleen cells were still capable of donor type Ig production after transfer, although in one experiment, a 50% reduction in donor type Ig was observed in the 1 week sample Similarly, the use of spleen cells from nude mouse donors (on CBA (Ig^a allotype) background) transferred into lethally irradiated C57BL recipients also resulted in Ig production As these four experiments all involved transfer into recipients possessing T cells the functional capabilities of which in this context may be radioresistant, we performed one cell transfer experiment with anti-θ treated BALB/c Ig^b spleen cells into homozygous nude BALB/c (Ig^a allotype) mice (of six backcross derivation from BALB/c, Holmes) Whereas 21 out of 21 normal BALB/c recipients had donor Ig levels between 10% and 40% 14 d later (mean 23 2), 7 out of 17 of the nude recipients failed to support any detectable donor Ig production, and the remaining 10 had similar values to the intact mice (range 9–40, mean 25 8%)

These experiments suggest that the level of Ig production by donor B cells is not markedly influenced by either the proportion of T to B cells present in donor spleen preparations, the possible subtypes of T cells present, or the absolute number of transferred T cells These results, however, do not deny the possibility that subpopulations of B cells in the spleen may be relatively dependent on help from T cells, and that in this

system of total Ig transfer without deliberate antigen injection, the past environmental exposure of the donor animal may influence the proportion of these B subtypes in spleen, and, for example, in experiment 1, the population dependent on T cells may be present to a relatively greater degree Resolution of this aspect is being approached by the use of defined B cell populations separated by the Los Alamos fluorescence-activated cell sorter

The results obtained in experiment 5 with transfer of anti-θ cells into nude recipients suggest that some nude mice may be incapable of supporting Ig transfer whereas others can do so It is generally considered that nude mice are devoid of T cells, although several recent studies[4], (Gutman, G, personal communication) suggest that this may not always be the case, and varying numbers of T cells may be found in the lymphoid tissues It may therefore be that this Ig transfer system has an absolute requirement for at least a minimal number of T cells (in either donor cells or host animal), and the 7 out of 17 nude recipients may represent this total T-deficient state Apart from this latter possibility, the present conclusion from these studies is that a large part of the Ig transfer is T-independent

We therefore investigated whether additional T cells would augment or suppress the Ig transfer The basic system was to use added thymus cells of mouse strain syngeneic to the recipient (in both allotype and histocompatibility) Numerous experiments of this type were performed with attention to several variables ratio of thymus to spleen cells, age of thymus donor, strain combination, time of assay post transfer, and effect of irradiation of the thymus cells

The effect of additional thymus cells on Ig transfer ascertained at several times after transfer is shown in Fig 1 Addition of 7-week-old thymus cells at a 1 1 ratio to spleen cells, and using either intact or irradiated (1,000 rad) thymus, resulted in a fourfold increase in donor Ig transfer at 7 d, declining to no difference at 4 weeks Over this period the absolute amount

Table 2 Effects on transferred immunoglobulin production of thymus cell addition to spleen cells

Experiment no *	Donor type immunoglobulin (% standard†)			Relative‡ increase	
	Control	With thymus cells	With irradiated thymus cells	Thymus 400/ thymus	Thymus 400/ control
1	0 6±0 1	2 1±0 4	5 5±1 4	2 6	9 2
2	6 0±1 0	41 5±3 1	36 1±5 1	0 9	6 0
3	18 0±7 2	60 0±12 0	61 0±10 0	1 0	3 3
4	0 8±0 3	0 6±0 2	1 4±0 2	2 3	1 8
5	—	18 5±4 8	35 2±4 5	1 9	—
6	1 3±0 2	0 3±0 1	4 8±0 8	16 0	3 7

*The conditions of transfer for each of the experiments in terms of several variables were as follows 1, Spleen donor C57BL/6 Ig^e, thymus donor 10 week C57BL, ratio thymus spleen 1 2, serum assay d 7 2, Spleen donor BABB-14, thymus donor 5 week BALB/c, ratio thymus spleen 1 1, serum assay d 14 3, Spleen donor C57BL/6 Ig^e, thymus donor 7 week C57BL, ratio thymus to spleen 1 1, serum assay d 14 4, As for 3 but 6 d serum assay 5, Peyer's patch lymphoid cell donor BABB-14, thymus donor 5 week BALB/c, ratio thymus to spleen 2 1, serum assay d 13 6, Spleen donor CB57BL/6 Ig^e, thymus donor 1 d old C57BL, ratio thymus to spleen 4 1, serum assay d 14 All recipients are syngenic to thymus donor Irradiated thymus cell suspensions all given 400 rad
†The results are expressed as percentage donor spleen type adult serum pool±s e mean
‡Ratio of donor type Ig levels for groups given spleen cells and irradiated thymus cells to either spleen cells alone or spleen and non-irradiated thymus

of donor Ig was increasing in the control mice, thus indicating a more rapid production of Ig in the thymus-added group, but reaching the same final level The addition of 7-month-old thymus cells had a less pronounced effect, indicating a possible change in the thymus cellular population at this age

A summary of six experiments is given in Table 2 and experiment 3 is depicted in Fig 1 The relative effect of added intact thymus cells assessed 1-2 weeks after transfer shows that in five experiments (control mice not available in number 5) augmentation of Ig transfer was achieved in three, with little change or suppression being observed in the other two In all six experiments, however, addition of irradiated (400 rad) thymus cells gave marked augmentation In four experiments, Ig production resulting from added irradiated thymus was greater than with added non-irradiated thymus

Further studies on the cellular specificity of this effect are in progress, although at present little augmentation has been observed with added bone marrow cells Two observations suggest that these results are indicative of alternative functions of subsets of T cells on the B cell system First, addition of 7-month-old or newborn thymus had relatively little augmentative effect, also indicating that the results are not nonspecifically related to some filler cell function which might be contributed by any added cell, and second, the increased relative effect of irradiated thymus to intact thymus is consistent with the thesis that a subpopulation of T cells can suppress Ig transfer, that is, perhaps analogous to suppressor T cells as described in other systems[5], and that this suppressor population is relatively radiosensitive in comparison to the helper T cell subset, an observation made previously[6,7] Also, the effect of radiation in distinguishing help from suppression was most pronounced with the newborn thymus, perhaps inferring the presence of a relatively greater proportion of suppressors at this age

These results may also infer that the transfer of Ig or antibody production by direct injection of marked thymus cells could be markedly dependent on the relative proportions of helper and suppressor T cells in the thymus cell suspension relative to the 1-2% of contaminating B cells found in most thymus cell preparations[8]

The use of the cellular transfer system of allotypically marked immunoglobulin is now being assessed as a model system for the analysis of the ontogeny of suppressor T cells and also, with appropriate allotype congenic NZB mice, in the role of suppressor T cells in spontaneous autoimmune disease The relatively minor effect of T-cell depletion on Ig transfer reinforces the previous suggestion[2] that inhibition of IgG transfer following anti-μ chain treatment is a result of inhibiting μ-bearing cells which otherwise were destined to switch to IgG secretion Further analysis of this latter point is being made by direct use of cells sorted on the basis of membrane-bound Ig

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T-cell dependence of immune response to hepatitis B antigen in mice

THE presence of hepatitis B surface antigen (HB_sAg) in blood is a marker of infection with hepatitis B virus (HBV) Persistence of the antigen in the blood of 'carriers' is a manifestation of proliferation of HBV in liver cells and this carrier state can be associated with various types of liver disease[1] In guinea pigs and chimpanzees, immunisation with HB_sAg in Freund's complete adjuvant (FCA) elicits a cutaneous delayed type hypersensitivity (DTH) response[2,3] It is believed that integrity of the thymus-dependent cell-mediated immune system in man is necessary for resistance to HBV, and that deficiency of cell-mediated immunity could predispose to development of the carrier state[4,5] We therefore investigated the contribution of thymus-derived cells to the immune response to HB_sAg in mice by immunisation of BALB/c mice, mice heterozygous for the nude mutant gene (nu/+), and congenitally athymic mice of the nu/nu strain, using purified HB_sAg

From the plasma of an adult asymptomatic carrier (subtype adw), HB_sAg was isolated by a combination of isopycnic banding and rate sedimentation using CsCl gradients[6] The purified HB_sAg was free of human serum components A reduced and alkylated form of HB_sAg (RA-HB_sAg) was prepared by treating purified HB_sAg (0 5 mg ml^{-1}) with an equal volume of 0 4 M 2-mercaptoethanol for 3 h at 37 °C followed by iodoacetamide in the dark at 4 °C for 1 h Purified HB_sAg was labelled with ^{125}I (carrier-free, Radiochemical Centre, UK) using chloramine-T as an oxidising agent[7], the specific activity was 27–35 μCi μg^{-1}

Four mice from each of the three groups (BALB/c, nu/+ and nu/nu) were injected subcutaneously in four sites adjacent to lymph nodes with 20 0 μg HB_sAg in 0 4 ml FCA The FCA (Difco) contained 0 5 mg ml^{-1} of *Mycobacterium butyricum*, and was supplemented with 4 mg ml^{-1} of *M tuberculosis* (H 37 RA, Difco) In addition four mice from each group were injected similarly with 20 0 μg RA-HB_sAg in 0 4 ml FCA After 18 d all 24 mice received an intracutaneous injection of 10 0 μg HB_sAg in saline in the left hind footpad as a challenge for a DTH response The next day the mice were killed, bled by cardiac puncture, and the spleen was removed and placed in Eisen's balanced salt solution The left hind footpad was amputated and placed in formalin for fixation before preparing sections for histological examination

Serum was tested under code, for antibody to HB_sAg by a haemagglutination assay[8] and for HB_sAg by a solid-phase radioimmunoassay (AUSRIA™)[9] A suspension of 1×10^7 single cells from each spleen was mixed with 1 0 μg ^{125}I-HB_sAg and tested for antigen-binding lymphocytes (ABL)[10] The slides were examined under code for ABL and scored as previously described[11], cells with 5–25 grains above background were scored as lightly labelled, and those with more than 25 grains as heavily labelled DTH reactions were assessed only microscopically, with histological sections of the foot-pads being examined under code The basis for scoring the intensity of DTH reactions was the degree of infiltration of cells of mononuclear type, for example 1+ represented minimal and mostly perivascular infiltration, and 4+ represented massive and diffuse infiltration throughout the section The results are shown in Table 1

In BALB/c mice and in heterozygotes the native HB_sAg induced high titres of antibody to HB_sAg Concurrent titrations on native sera and sera treated with 2-mercaptoethanol

Table 1 Immune response to HB$_s$Ag and RA-HB$_s$Ag in athymic *nu/nu*, heterozygote and normal BALB/c mice

Groups of mice (four per group)	Immunised with	DTH response to HB$_s$Ag*	Mean ABL per 1,000 lymphocytes†			Antibody to HB$_s$Ag (geometric mean titre)‡
			H	L	Total‡	
nu/nu	Native HB$_s$Ag	−, −, +, 2+	0 9	1 9	2 8	0
nu/nu	RA-HB$_s$Ag	+, +, 2+, 3+	0 2	1 7	1 9	0
nu/+	Native HB$_s$Ag	2+, 2+, 3+, 3+	5 5	12 0	17 5	75
nu/+	RA-HB$_s$Ag	2+, 3+, 4+, 4+	2 3	5 5	7 8	45
BALB/c (+/+)	Native HB$_s$Ag	2+, 4+, 4+, 4+	3 9	12 2	16 1	75
BALB/c (+/+)	RA-HB$_s$Ag	3+, 4+, 4+, 4+	2 9	9 6	12 5	53

*Presence and degree of DTH response to HB$_s$Ag were assessed by histological evidence of mononuclear cell infiltration 24 h after intracutaneous challenge, neutrophil infiltration was negligible in all sections
†Antigen binding lymphocytes per 1,000 spleen lymphocytes, H, heavily binding and L, lightly binding (see text) As controls, counts of splenic ABL for HB$_s$Ag in non-immunised *nu/nu*, *nu/+* and BALB/c mice were respectively 0 9, 2 6 and 1 6
‡Differences were significant (*t* test) between group *nu/nu* and other groups for counts of ABL (*P* < 0 005) and antibody (*P* < 0 02)

gave comparable results, indicating that the class of antibody to HB$_s$Ag was IgG Moreover, there were high counts of ABL in the spleen, and a pronounced DTH reaction in the skin Immunisation with RA-HB$_s$Ag gave comparable results for antibody, ABL, and DTH On the other hand, the immune response to HB$_s$Ag and RA-HB$_s$Ag in athymic *nu/nu* mice was weak or absent, as judged by no detectable antibody, minimally raised counts of ABL in the spleen, and lower DTH responses Tests for HB$_s$Ag in serum were uniformly negative

The above findings indicate that in mice, HB$_s$Ag elicits strong DTH responses, and is highly thymic-dependent with regard to requirement for T-cell help for a humoral antibody response The presence in athymic *nu/nu* mice of ABL in the spleen is consistent with the interpretation[12] that such ABL are B cells Although RA-HB$_s$Ag induced only DTH but no humoral immune response in guinea pigs[2], in mice it induced both types, this observation supports the finding[13] that the immune response to RA-HB$_s$Ag varies in different species Thus, it could be suggested that the carrier state for HB$_s$Ag in man is akin to a state of tolerance consequent on failure of T-cell recognition of immunogenic components of this antigen

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Human lymphocytes recognise mouse alloantigens

H-2 LD antigens[1] of the mouse major histocompatibility complex and the lymphocyte-activating determinants of the *Mls* (ref 2) locus induce vigorous lymphocyte proliferation and enhance generation of cytotoxic effector cells in allogeneic combinations (ref 3 and D J Schendel, and F H Bach, unpublished) These antigens do not serve well as targets for cell-mediated lympholysis (CML)—a function which in the mouse is performed by the classical H-2 determinants, the SD antigens[4,5] A similar differential function of LD and SD antigens is found in human allograft reactions *in vitro*[6]

Although the polymorphism of the LD antigens clearly is extensive, it is possible that the difference between the LD alleles of two animals of the same species is only a few amino acid substitutions and not enough to allow effective recognition by cytotoxic effector cells It might be expected that with increasing phylogenetic distance there would be a greater difference between the LD alleles of two animals, such that effector cells sensitised against the LD antigens from another species might show positive cytotoxicity towards these antigens

Using human lymphocytes immunised *in vitro* against mouse spleen cells, we confirmed that xenogeneic effector cells are specifically cytotoxic to target cells of the same H-2 type as the sensitising strain[7,8] and found no evidence of differential recognition of any non-H-2 target[9] (see also Fig 1 and Table 1) In this report we describe the fine specificity of H-2 target antigens in xenogeneic combinations The major conclusion is that in xenogeneic as in allogeneic combinations, cytotoxicity is mainly directed towards the H-2 SD antigens, whereas the LD antigens do not function as targets, or do so only poorly

The H-2 complex is divided into four regions K, I, S and D, genes of the K and D regions (H-2K and H-2D loci) determine the H-2 SD antigens, the strong LD antigens are products of genes in the I region Human lymphocytes were sensitised to each of the four strains B10 A(4R), B10 A(2R), AQR, and B10 T(6R), abbreviated 4R, 2R, AQR, and 6R, respectively, and their cytotoxic potential was measured on target cells from each strain The genetic constitution of these strains enables us to assay the importance of the K and D regions versus the I and S regions as targets for cytotoxic effector cells (Fig 2) If the SD antigens of the K and D regions are the prime targets for xenogeneic as for allogeneic effector cells, and the LD antigens do not serve well in this capacity, we would expect that human lymphocytes sensitised to 4R and 2R are equally and highly cytotoxic to targets of these strains and give little or no killing on target cells from AQR and 6R, as a corollary, effectors sensitised to AQR or 6R would give equal and high killing on targets of these two strains and only low killing on targets from 4R or 2R As Fig 1 shows, this is clearly the case

We have carried out nine experiments of this type One was excluded because there was no cytotoxicity on any target and

Table 1 Importance of products of K+D against I+S regions of H-2 as targets for xenogeneic effector cells—summary of eight experiments

Target cells*	Effectors sensitised to	H-2 regions shared with target	Cytotoxicity† (%)		P‡
			Median	Range	
1R or 2R§	4R	K, I-A, D	11 55	2 7–29 6	>0 05
	1R or 2R§	K, I, S, D	11 25	2 7–44 4	=0 01
	AQR	I, S	3 1	−2 1–16 2	>0 05
	6R	—	1 7	−0 4–20 0	=0 025
	H‖	—	0	0	
AQR	6R	K, D	13 4	3 8–47 5	>0 05
	AQR	K, I, S, D	12 3	3 8–43 8	<0 025
	1R or 2R§	I, S	1 2	−6 8–31 1	>0 05
	4R	I-A	3 1	−5 8–44 3	>0 05
	H‖	—	0	0	

* Activated with LPS (three experiments) or PHA (five) The results have never indicated a difference between the two kinds of targets, and they have been pooled
† Cytotoxicity (%) with 140 (one experiment), 80 (one), or 70 (six) effector cells per target cell Where several ratios were tested in one experiment, 70 1 was used in this analysis
‡ Wilcoxon's signed rank test (one-tailed)
§ Four experiments with each strain gave the same results and have been pooled
‖ Autologous controls Seven different donors were used
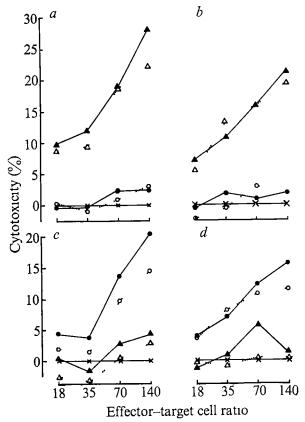
Fig. 1 Human effector cells cause specific ^{51}Cr-release from mouse target cells carrying the same H-2 SD antigens as the sensitising strain Targets *a*, 4R, *b*, 2R, *c*, AQR, *d*, 6R Human lymphocytes (12×10⁶) from donor H, purified by Ficoll–Hypaque flotation[10], were cultured with mitomycin C-treated[11] stimulating cells, 24×10⁶ autologous lymphocytes, Hm, or 35× 10⁶ mouse spleen cells, in upright Falcon No 3013 flasks in RPMI 1640 with 7 5% heat-inactivated foetal calf serum, penicillin, and streptomycin After culture for 6 d at 37 °C in 5% CO₂ in humidified air and change of 25% of the medium on days 2 and 4, the viable cells were recovered and mixed at the indicated ratios with 10⁴ ^{51}Cr-labelled target cells and incubated for 3 h according to Alter *et al*[4] The target cells were mouse spleen cells stimulated 2 d previously with LPS

the remaining eight are summarised in Table 1 Two features in particular should be noted First, human lymphocytes sensitised against the I and S regions of the target do not give significantly higher cytotoxicity than lymphocytes sensitised to a different H-2 haplotype (lines 3 and 4, lines 8 and 9) Second, equal killing is obtained when the effector cells are sensitised to the whole H-2 complex or only to the K and D regions of the target (lines 1 and 2, lines 6 and 7) We have obtained similar results on target cells stimulated with LPS (presumably B cells[13]) and PHA (presumably T cells[13]), suggesting that the failure to detect cytotoxicity directed against products of the I and S regions is not an artefact resulting from the tissue distribution of these antigens Table 1 also shows that there is very little difference between the killing by effector cells sensitised to the non-H-2 antigens of the target and by non-sensitised, cultured cells (lines 4 and 5, lines 9 and 10) If species-specific antigens exist in the man–mouse combination, they must, therefore, play a very minor role for cytotoxicity

In conclusion, we have shown that of all the antigens expressed on a mouse lymphocyte surface, human effector cells primarily recognise products of the H-2 K and D regions, presumably the SD antigens themselves, which only constitute a few per cent of all surface proteins[14]

The narrow specificity of cytotoxic effector cells for SD antigens in an allogeneic combination does not seem, therefore, to be the result of a limitation in the number of antigenic determinants which can be recognised, since it is possible to get good cytotoxicity to any number of new determinants presented by xenogeneic cells or generated by chemically modifying syngeneic cells[15] But in both situations it is primarily the products of the H-2 K and D regions which function as targets This suggests that there are important biological

Fig 2 H-2 haplotypes of the strains used in this study 1R and 2R are identical for all the marker loci of H-2 and probably only differ for a chromosomal segment between S and D They have therefore been used equally in these experiments 4R, 1R/2R, and 6R are congenic resistant partners carrying different H-2 haplotypes on the same B10 background AQR was backcrossed four times to C57BL/10 before it was established as an inbred line, it therefore carries a considerable portion of the B10 genome, and studies have shown that it is the H-2 complex of AQR which is most important in allograft reactions with B10 congenic strains[1] All the strains are as a first approximation considered identical except for their H-2 complexes The pairs 4R-1R/2R and AQR-6R are SD-identical, but differ in the I and S regions, whereas 1R/2R-AQR are SD-different but identical for the I and S regions

requirements for target antigens, such as their chemical nature, their density on the cell surface[16] or rate of turnover, which are best fulfilled by the SD antigens These findings raise the question whether tumour-specific antigens can be recognised by cytotoxic effector cells unless they are associated with or modify the SD antigens of the major histocompatibility complex

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Properdin factor B and histocompatibility loci linked in the rhesus monkey

IN man, mouse, monkey and dog the major genetic loci for serologically defined (SD) and mixed lymphocyte reacting (MLR) histocompatibility determinants comprise a closely linked gene complex—the major histocompatibility complex (MHC)—on a single chromosome[1-4] Genes controlling immune response in mice are located within the MHC[3] and preliminary data for the rhesus monkey suggest that this species also has these genes within or adjacent to the MHC[5,6] The preservation of the gross structure of this chromosome

region during a long period of mammalian evolution is of considerable biological interest In addition, the demonstration in the mouse of genetic linkage between MHC and resistance to virus infection[7] and leukaemogenesis[8] and the recognition of association between certain HL-A haplotypes and susceptibility to disease in the human indicates that the MHC may be an area of potentially great practical significance in addition to its role in transplantation

Genetic polymorphism of properdin factor B[9] is linked to HL-A in man[10], and the recognition of factor B polymorphism in the rhesus monkey[11] provided the opportunity to test for such linkage in this species Our results suggest close linkage between the *Bf* (factor B) locus and various genetic systems known to be located in the MHC or RhL-A complex of rhesus monkeys[12,13]

Animals studied were part of the breeding colony of the Primate Center TNO, Rijswijk Sera from 76 members of 22 full sibships, which had been sired by one of four males were investigated RhL-A(SD) haplotypes (there are two closely linked SD loci) were determined on the 22 mothers and four fathers (who were unselected and, as far as can be determined, unrelated) and the 76 progeny as previously described[14] Bf phenotypes were determined[11] without knowledge of SD haplotypes in the 76 progeny, three male and 20 female parents Two common Bf types (Bf F and Bf S) and four rare types (Bf G1, Bf G2, Bf S1 and Bf S2) have been recognised[14] and all except Bf G2 and Bf S2 were encountered in these pedigrees Three of the six matings of male No 381 (Bf SS) were uninformative two with a homozygous female and one with a female of unknown type which produced three homozygous progeny Data from the 22 sibships examined are presented in Table 1 Nineteen informative matings include those where maternal or paternal Bf types were deducible from data of multiple offspring In these matings at least one parent was heterozygous for *Bf* genes and the data showed that where a particular *Bf* gene could be followed from parent to offspring it was accompanied by the same RhL-A(SD) haplotype The preliminary assumption made from this observation is that the *Bf* and SD loci lie close together on the same chromosome, that is they are linked If this is the case, chromosomal crossing over between *Bf* and SD loci during meiosis would disrupt this pattern of linkage (if an odd number of crossovers occurred), producing recombinants There were only two certain recombinants, both involving maternal chromosomes In the mating male No 598×female No 832, the gene *Bf^s* of the female was accompanied by the SD d haplotype in AD and BM and its absence with the c haplotype in CN In DS it appeared with the c, however, indicating that DS represented a maternal recombinant In the mating male No 599×female No 493, AX was a maternal recombinant

Lod (logarithm of the odds) scores were calculated in informative families by standard techniques[15] The lod score in males was 10 1 for a recombination fraction (θ) of zero and in females had a maximum value of 5 6 (θ = 0 04) so that the probability of linkage is very high In the total population the maximum lod score was 15 1 (θ = 0 02) and the odds in favour of linkage as opposed to a chance association exceed 10^13 to 1 Corrections for prior probabilities have not been included Three of the four males were heterozygotes for Bf and the most likely phase (whether, in a given animal, specific SD and *Bf* genes are on the same or different chromosomes) can therefore be deduced in one mating Lod scores in males would be even higher if this information were taken into account So far no paternal recombinants have been detected but a higher incidence in females is frequently found in linkage analysis[16] Since the most likely recombination fraction in the pooled data is 0 02, this, if correct, would indicate a map distance of 2 centimorgans between *Bf* and the region coding for serologically defined antigens of RhL-A No evidence for linkage disequilibrium has been found in these studies

In each of two matings with male No 599, one paternal recombination between first and second SD series of RhL-A

Table 1		Genotyping for serologically defined (SD) antigens of RhL-A and properdin factor B (Bf) genes in rhesus monkey families									
Parents and offspring	SD* haplotype	Bf type	Parents and offspring	SD haplotype	Bf type	Parents and offspring	SD haplotype	Bf type	Parents and offspring	SD haplotype	Bf type
♂381	ab	SS	♂598†	ab	(S1F)	♂599	ab	FS	♂600	ab	SF
♀584	cd	SS	♀834	cd	FS	♀581	cd	FF	♀669	cd	SS
V	bd	SS	UU	bc	FF	G	bc	SF	AC	ad	SS
JJ	bc	SS	BD	bc	FF	GG	bc	SF	BI	ad	SS
AV	bc	SS	CS	ad	S1S	AG	ac	FF	CR	ad	SS
BU	bc	SS	EK	bc	FF	CF	ac	FF	EL	bc	FS
CZ	bc	SS				DV	bc	SF	FS	bc	FS
♀432	cd	G1F	♀730	cd	SG1	♀1115	cd	SS1	♀603	cd	FS
N	ac	SG1	FF	bd	FG1	CL	a/bc	FS	T	bd	FS
AK	bd	SF	BT	ac	S1S	EF	bd	SS1	DF	bd	FS
CP	ad	SF	DA	ad	S1G1	GZ	ac	FS	FO	bd	FS
FN	ac	SG1							GY	bc	FF
♀852	cd	FS	♀832	cd	FS	♀498	cd	SF	♀597	cd	FF
AH	ac	SF	AD	bd	FS	NN	ac	FS	AB	bc	FF
BL	bd	SS	BM	ad	S1S	AQ	ac	FS	BF	bc	FF
DN	ac	SF	CN	ac	S1F	ED	a/bd	FF	CG	ad	SF
EV	ad	SS	DS	bc	FS←				DW	ac	SF
♀594†	cd	(S)	♀589†	cd	(SS1)	♀590	cd	SS	♀429	cd	G1F
YY	ad	SS	Z	ac	S1S	CV	bd	SS	XX	bc	FG1
BJ	bd	SS	AE	ad	S1S1	EI	ad	FS	BG	ac	SG1
FU	bd	SS	BP	bc	FS	GD	bd	SS.			
						HG	ac	FS			
♀494	cd	SF	♀728	cd	G1S	♀493	cd	G1F			
L	ac	SS	KK	ad	S1S	AX	bc	SF←			
AA	bd	SF	AS	bd	FS	CK	bc	SG1			
AM	ad	SF	EP	ac	S1G1	DM	bc	SG1			
CY	ac	SS									
♀324	cd	S1S1	♀306	cd	FS	♀926	cd	SS			
CU	bc	SS1	SS	bc	FF	DC	bc	SS			
EM	ac	SS1	BK	ac	S1F	FJ	ac	FS			

*By convention, a and b are paternal, c and d maternal haplotypes, for details of SD haplotyping, see ref 12
†Animal dead
( ) Bf type 'deduced' when possible Arrows indicate suggested recombinants, see text

was encountered[12] In each case, the Bf type accompanied the first segregant series SD type, suggesting that the Bf locus lies on the first series side of the complex Unfortunately, matings in which recombination occurred between the SD region and the major MLR locus of RhL-A were uninformative for Bf linkage MLR, however, probably also maps on the side of the first RhL-A(SD) locus [17] and if this is confirmed, the following reasoning can be applied AX is a maternal recombinant (Table 1), being of different Bf type but SD identical to her siblings CK and DM In MLR, AX also fails to stimulate or be stimulated by her siblings, indicating lack of recombination between SD and the major MLR loci[17] DS has also been shown not to be a recombinant between the SD and major MLR loci These observations can best be explained by placing the MLR locus between Bf and SD or by placing SD between Bf and MLR Since both Bf and MLR seem to be on the first segregant series side of the rhesus SD complex, the former situation (Fig 1) is the more likely Preliminary data in humans (Ch Rittner, personal communication) indicate that MLR is between SD and Bf in this species

In the mating male No 598 × female No 834, EK has been found to be a recombinant between SD and Ir-GA (a gene controlling immune responsiveness to the linear synthetic polymer of *l*-glutamic acid and *l*-alanine)[6,18] Since EK does not show recombination between Bf and RhL-A(SD), it seems that either the Bf locus lies between SD and Ir-GA or the SD region lies between Bf and Ir-GA A possible situation, suggested by available data, is summarised by Fig 1

The HL-A complex of humans lies on the sixth autosome[19,20] and includes the LA and FOUR series (SD) which are closely linked to MLR, Bf, as well as to PGM₃[16], ME1 and IPO-B[19] There may also be a human Ir (immune response) region in the MHC A region showing striking homology to the human MHC exists in the rhesus monkey, and appears to have been conserved through much of mammalian evolution Bf, apparently closely associated with the MHC, will provide a convenient reference marker for other linkages

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Fig. 1 Possible map of the chromosomal region containing the major histocompatibility or RhL-A complex in the rhesus monkey Ir-GA and Ir-GL, immune response genes Bf, properdin factor B structural locus SD, two loci for serologically defined histocompatibility antigens MLR, major locus for mixed lymphocyte reactivity Evidence placing MLR nearer to first segregant SD series (SD₁) remains tentative Note that Ir-GA has been placed on the outside of the complex but further localisation is not possible

(Ir-GA) *Bf* MLR Ir-GL   SD₁   SD₂   (Ir-GA)

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Follicle stimulating hormone enhances attachment of rat testis cells in culture
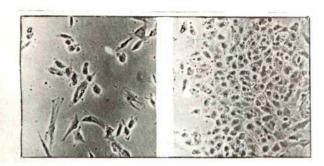
EXTENSIVE *in vivo* experiments indicate that mammalian spermatogenesis is under the regulation of pituitary gonadotrophins. The cellular sites and mechanisms by which spermatogenesis is regulated by gonadotrophic hormones, however, are poorly understood. Means has shown *in vivo* that follicle stimulating hormone (FSH) stimulates several cellular processes[1,2]. So far, however, no one has shown an *in vitro* effect of FSH on the testis. Several reports have indicated that hormones or hormone mediators alter cell properties in culture. In particular, testosterone was found to cause morphological changes in CHO cells[3] and cyclic adenosine 3′,5′-monophosphate has been shown to affect the adhesive properties of L-929 (ref. 4) and BHK (ref. 5) cells. We have been studying the growth of testis cells from the 10-d-old rat in culture and report here our observations on the stimulation of testicular cell attachment to culture dishes by follicle stimulating hormone.

Cells from 14 testes were dissociated by continuous agitation in 0.1% trypsin (Gibco, 1:250) for 50 min in a trypsinising flask. The tissue-free supernatant was collected at 10-min intervals and diluted with 8% foetal calf serum (1:1). Cells were collected by centrifugation at 1,000g for 10 min and suspended in NCTC-135 media (Gibco) containing 10% foetal calf serum.

Isolated testis cells (5×10^5 per dish) were incubated for 24 h in 35-mm dishes (Falcon) in 2 ml of NCTC-135 with foetal calf serum, (10%), penicillin (100 U ml^{-1}), and streptomycin (100 μg ml^{-1}) at 31.5 °C in an air–CO$_2$ atmosphere (95:5). Culture dishes contained either FSH (NIH-FSH-S$_8$), human chorionic gonadotrophin (HCG, Ayerst 531 U mg^{-1}), or bovine serum albumin (BSA). In dishes without FSH, individual fibroblast-like cells were seen on the bottom of the dish (Fig. 1*a*) whereas in the dishes containing FSH, colonies containing round, tightly-packed cells were seen (Fig. 1*b*).

After incubation, the dishes were washed twice with 2 ml of phosphate buffered saline to remove unattached cells, fixed for 12 h in 10% formalin, and stained for 5 min in haema-

Fig. 1 *a*, Phase contrast photomicrograph of cells growing in culture without FSH. *b*, Phase contrast photomicrograph of a cell colony growing in culture with FSH ($\times 160$).
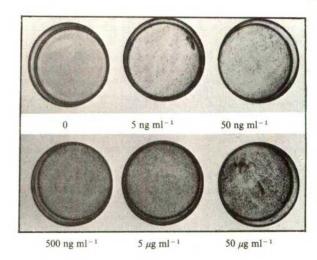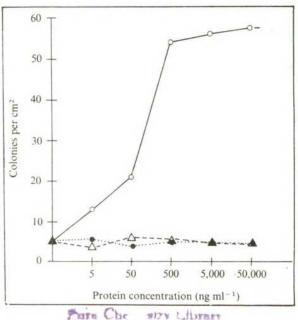
Fig. 2 Photograph of haematoxylin-stained colonies in dishes treated with various concentrations of FSH after 24 h in culture.

toxylin. Examination of stained colonies on the bottom of the dish indicated a dose-dependent (0–50 μg ml^{-1}) stimulation of colony number by FSH (Fig. 2).

To examine more closely the effect of FSH on colony formation, cultures were treated with FSH, HCG or BSA at 5–50,000 ng ml^{-1}; triplicate points were run at each dose in three experiments. Stained dishes were then placed on a 4×4-cm grid and the colonies in 5-cm^2 areas chosen from a random number table were counted at $\times 6$ magnification under a dissecting microscope. Colony counts are summarised in Fig. 3, and show a clear dose-dependent effect of FSH ($P < 0.01$, analysis of variance) on colony number while HCG and BSA had no significant effect. Furthermore, in subsequent experiments, HCG had no effect on colony number at doses up to 5 mg ml^{-1} (2,655 IU).

In the same experiment the effect of the various treatments on the size (number of cells) of the colonies was determined.

Fig. 3 The effect of FSH (○), HCG (●) and BSA (△) on colony density after 24 h in culture. Dishes were stained with haematoxylin and the colonies in 5 cm^2 chosen at random on the bottom of the dish were counted. The average of the five determinations for three dishes represented one experimental value. The points are an average of the three experiments.
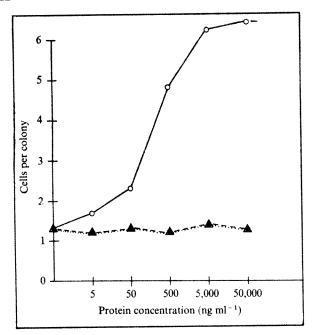
Fig. 4 The effect of FSH (○), HCG (●) and BSA (△) on colony size after 24 h in culture. Dishes were stained with haematoxylin and cells per 100 colonies were counted at ×200. The average number of cells per colony for three dishes represented one experimental value. The points are the average of three experiments. Note: △ and ● are superimposed.

Cells were counted at ×200 magnification on 100 colonies on the bottom of the dish. As with colony density, colony size increased with FSH but not with BSA or HCG (Fig. 4). As before, the effect of FSH was dose-dependent and analysis of variance indicated a highly significant treatment effect ($P < 0.01$).

The results indicate that FSH has a stimulatory effect on some testicular cell in culture. Our preparation of testes cells from the 10-d-old rat contained more than 60% preleptotene spermatocytes; however, these cells do not attach to culture dishes[6] and therefore do not seem to have been the cells stimulated with FSH. Extensive electron microscopic examination of the cells in colonies indicated that they were not germinal cells and were a single cell type; however, an exact identification of the cell type involved would be premature at this time.

Our observation of an action of FSH on a testicular cell *in vitro* agrees with the observations that FSH (1) stimulates RNA synthesis[1] and protein kinase activity[2] and (2) is involved in the initiation of spermatogenesis[7] in the rat testis *in vivo*. The observation that HCG does not stimulate colony formation suggests that the *in vitro* effect may be, like the *in vivo* effect, hormone specific. These findings demonstrate an action of FSH on a discrete testicular cell *in vitro* and should provide new insight into hormonal and cellular interactions involved in spermatogenesis.

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Ultrastructure of muscle spindles in dystrophic mice

ALTHOUGH the pathological changes in muscular dystrophy are commonly attributed to a primary abnormality of the muscle fibres, there is evidence to suggests that mucular dystrophy may be attributable to a neuronal abnormality[1]. This has become known as the 'neural hypothesis'. Dystrophic mice (Bar Harbor 129 REJ *dy/dy*) provide a useful model of muscular dystrophy for experimental study. In spite of the early onset of abnormal reflexes in muscular dystrophy, the richly innervated sensory organs, muscle spindles, have received relatively little attention[2,3]. Optical microscopical studies have indicated that spindles in dystrophic mouse muscles are essentially unaffected[4], although in some human diseases they seem to undergo minor changes[5]. This study was undertaken to establish whether there are ultrastructural changes in spindles of dystrophic mice which are detectable only by electron microscopy.
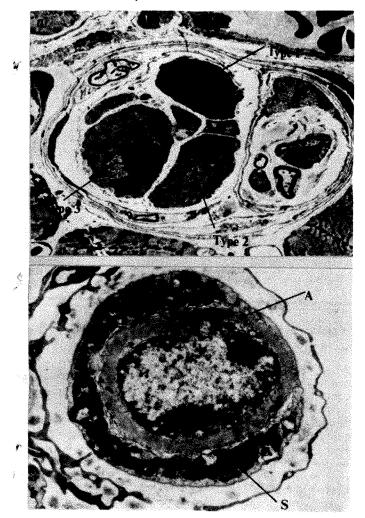
In dystrophic muscle each spindle was found to possess a prominent periaxial space containing a characteristic flocculent precipitate. The periaxial space was bounded by a capsule containing up to six layers of perineural epithelial cells. Each perineural epithelial cell contained numerous pinocytotic vesicles. The capsule enclosed the muscle fibres of the spindle for most of their lengths except close to their insertions into perimysium. Capillaries were never seen to penetrate the capsule and enter the periaxial space.

The dystrophic spindles contained an average of three to eight intrafusal fibres. No spindle contained less than three intrafusal fibres. Nuclear bag fibres contained up to three nuclei and a peripheral rim of myofilaments in each equatorial cross section and could be clearly differentiated from the narrower and shorter nuclear chain fibres, which contained singly occurring central nuclei. Both types of intrafusal fibre contained numerous myofilaments in their polar regions. Nuclear bag fibres tended to contain confluent masses of myofilaments interspaced with occasionally occurring mitochondria of variable size. In a few nuclear bag fibres the mitochondria were of more regular size and more evenly dispersed throughout the fibre cross section. These two types of intrafusal fibre correspond to those designated types 2 and 3 in other species[7]. Nuclear chain fibres contained groups of myofilaments which formed discrete myofibrils often separated by numerous mitochondria. In both nuclear bag and nuclear chain fibres the number of myofilaments was markedly reduced in equatorial compared with polar regions. Dilated terminal cisternae of the triads typical of those found in other species[8] were also found.

Satellite cells were found lying between the basement membrane of the intrafusal fibres and their plasma membranes. They seemed inactive because their relatively small amounts of cytoplasm contained few ribosomes and a little rough endoplasmic reticulum. All the intrafusal fibres examined received a direct sensory innervation without intervening basement membrane. Two motor-nerve endings were also observed. Their primary synaptic clefts contained a continuous layer of basement membrane which extended into the relatively few secondary synaptic clefts that occurred.

None of the spindles in dystrophic muscles was seen to contain any ultrastructural abnormality and did not differ significantly from the spindles in the control muscles. These findings have important implications for the 'neurogenic hypothesis' of muscular dystrophy. If that hypothesis is true then changes in the richly innervated intrafusal fibres may be expected. Consequently, at least a modification of the hypothesis must be proposed. Several possibilities exist; for example, either there is a selective sparing of the sensory or γ-motor supply of

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Fig. 1 *a*, Transverse section across the polar region of a muscle spindle in a clinically dystrophic mouse. Three types of intrafusal fibre can be differentiated. Type 1 fibres (nuclear chain) contain many large mitochondria; type 2 fibres (nuclear bag) contain intermediate numbers of evenly scattered mitochondria; type 3 fibres (nuclear bag) contain a few small mitochondria. ($\times 2,300$). *b*, Transverse section across the equatorial region of the nuclear chain (type 1) fibre seen in (*a*). A primary sensory ending (S) contains numerous mitochindria and partly encircles the intrafusal fibre. The cross sectional area (A) which contains myofilaments in this region of the fibre is much smaller than in the polar regions. ($\times 12,000$). Specimens of soleus and extensor digitorum longus muscles were obtained from six clinically affected Bar Harbor 129 REJ *dy/dy* dystrophic mice and from six nonlittermate controls, and prepared for electron microscopy as described previously[6]. Eleven spindles were found in dystrophic muscle and eight in control muscle. Ultrathin sections were cut from these spindles at intervals of 25–50 μm along most of their lengths. A few spindles were also examined in longitudinally cut sections.

the spindles or, alternatively, the intrafusal fibres may have acquired a high resistance to the supposed neurogenic influence. In support of the latter, in many human muscle diseases, apart from an apparent disuse atrophy, the intrafusal muscle fibres usually seem to be relatively unaffected[9,10]. Although the relevance of such findings in dystrophic mice to the pathogenesis of human muscular dystrophy is uncertain, studies on human dystrophic spindles could be of considerable interest.

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Squint and the development of binocularity in humans

IN the past ten years it has been shown that binocularity[1], orientation sensitivity[2,3], ocular dominance[4] and disparity[5] of cortical neurones can be modified by experimental procedures in kittens during the sensitive period (3–14 weeks of age).

In 4-week-old kittens which had one eye closed for 6 d the percentage of binocular cells decreased to 9% from 80–90% in normal controls, but closing one eye for 2 months in 4-month-old kittens did not affect the relative number of binocular and monocular cells[6]. In humans, according to clinical experience, binocular vision is not fully established at birth and develops to full maturity during the first four to five years of life[7].

If neurones of the visual cortex need to be excited by identical or nearly identical stimuli from both eyes during the critical period in order to develop binocularity, a squint during this period should result in a smaller number of cells than normal with binocular input. Since binocular neurones of the visual cortex are an essential prerequisite for binocular single vision and depth perception[8], a lack of such cells will result in a failure of real binocular single vision after correction of the squint. This is also supported by clinical observations which indicate that the later a squint is acquired the more likely it is that binocular vision will be restored after a successful operation[9].

We have tried to correlate these clinical observations with quantitative measurements of interocular transfer in children whose squint had developed at different ages but was corrected by surgery. This was done with the assumption that interocular transfer may be a reliable indicator for the binocular input to visual cortical neurones. A simple test of this kind is the interocular transfer of the tilt after-effect described by Gibson[10]. If a subject fixates a grating tilted 10° anticlockwise away from the vertical for some minutes, he will make a systematic clockwise error when asked to adjust the same grating in the vertical position. The same systematic error is found, though to a smaller degree, if one eye is trained and then adjustment of the grating is done with the other, untrained eye (binocular transfer). Patients with strabismus have a smaller after-effect transferred from one eye to the other than subjects with normal vision[11].

Twelve children, 5.10 to 15.2 years old, served as subjects. Nine of them (mean age 9.8 yr) had been patients of the Göttingen eye hospital and had suffered from strabismus concomitans convergens alternans in early life. They had been operated on at the age of 3–5 yr (mean 4.1 yr). Preoperation squinting angle was between 12° and 40° (mean 23°). Binocular interaction was tested on the synoptophor, visual acuity with the Pflüger-hook. The Worth test and the Maddox cross test were also applied. The Gibson transfer phenomenon was then tested on the synoptophor. Three children with normal vision (mean age 5.9 yr) served as control subjects.
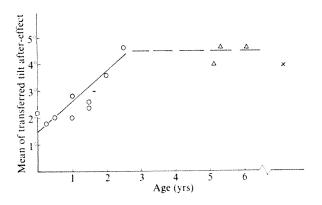
A vertical light bar on a black ground was adjusted five times for each eye and the angular error measured. This was of the order of $\pm 0.5°$. The subject then adapted monocularly for 2.5 min to a grating orientated 10° anticlockwise to the vertical. The bar had the same width as the lines of the grating. Immediately after this adaptation period the single bar, set at 5° clockwise to the vertical was shown to the untrained eye. The experimenter slowly rotated the single line and asked the subject to say 'stop' when the line appeared vertical to him. Normal children indi-

cated that the bar appeared vertical when it was still tilted about 4–5° from the normal. This value will be called transferred tilt after-effect (TTA). After readaptation for 45 s the test was repeated twice for each eye. The typical error between successive measurements was less than ±0.3°.

The age at which the squint first appeared was noted in the hospital records and the parents were also asked. Figure 1 shows that the TTA is smallest (1–2°) in children who had a squint from birth. It increases steadily the later the squint started. A child who had started squinting at the age of 2.6 yr showed no difference in TTA compared with subjects with normal vision. The TTA values shown on the ordinate of Fig. 1 are the means of the TTA values from the right to the left and from the left to the right eye.

Only 3 children were used as controls. Their TTA values were the same as those known for normal subjects in other investigations[12], so that further controls were not necessary. Ophthalmological assessment of binocularity closely corresponded to these results. The children who had started squinting at birth had simultaneous perception only (stage 1 of binocularity). Those who had acquired their squint at 0.6–1 yr could fuse images (stage 2), and those whose squint began at 1.6 yr or later could perceive depth (stage 3).

Fig. 1 Binocular transfer of tilt after-effect in operated strabismic children, whose squint developed at different ages. Abscissa: ○, age of onset of squint in patients; △, age of control children at which the measurement was done. Ordinate: mean error of adjustment of vertical line with one eye after exposure of the other eye to a grating tilted 10° away from the vertical for some minutes (transfer of tilt after-effect). ×, Control values from Campbell and Maffei[12].

Thus the later a squint began in the child the higher was his mean interocular transfer angle, and the better his binocular vision. If the squint started by the age of approximately 2.6 yr, normal interocular transfer was possible. These findings indicate that at an age of about 2.6 yr, binocular functions of the visual cortex as measured with the TTA are well enough established to be restored by correction of a squint. The age of about 2–2.6 yr may therefore be considered the end of the critical period for the development of binocular vision in humans.

The fact that the interocular transfer has never been found to be zero could indicate either that at least some binocular cortical neuronal connections develop independently of binocular vision during early life time, or that a post-operation training effect took place. Orthoptistic training is given to children until they are nine years old.

The good correspondence between the TTA results and the clinical assessment of binocularity suggests that the TTA is a good indicator of binocular functioning of the visual cortex. It may be directly related to the number of binocular units in the visual cortex as also suggested by earlier investigations[11,13]. As it is a simple test it could be included in clinical testing of binocular functions.

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Visual acuity development coincides with the sensitive period in kittens

AT about ten days of age when a kitten's eyes open, synaptic connections of its visual system are partly laid down[1]. They are completely developed during the sensitive period[2] which lasts from about three weeks of age to three months. During this period of plasticity the formation and modification of synaptic connections respond to features in the visual environment[3-6]. Both the original and subsequently developed connections may be disrupted or modified by environmental factors during the sensitive or critical period with a sensitivity that peaks at about the fourth week and gradually drops off[2].

Conventional (psychophysical) visual acuity as well as its electrophysiological acuity correlate, obtained by pattern-dependent visual evoked potentials, must also develop in the kitten as a result of the organisation of synaptic connections. (Visual evoked potential acuity measurements are based on electrical processing in the visual cortex, although it is possible that they may reflect retinal and geniculate processing resolution before cortical processing takes place.) Our goal was to measure visual acuity in cats and kittens of various ages to determine the time course of its development.

Visual evoked potential acuity measurements were made on nine kittens and three adult cats, all raised in a normal visual environment. The data were obtained from the two eyes separately in each of four kittens and in one eye in the rest of the kittens and cats, providing fifteen data points in all. A horizontal rather than longitudinal time study was dictated because stable electrodes were difficult to maintain through the thin, growing skull of the kittens. Following the method of Rose *et al.*[7], stainless steel screw electrodes were positioned just through the skull over the visual cortex and, with dental acrylic cement, were encased in a small electric connector on the head. The implantation of electrodes yielded excellent potentials with a much better signal to noise ratio than did surface electrodes.

The responses were evoked by the virtually instantaneous reversal of the light and dark stripes of a bar grating pattern by an optical electromechanical shaker. A constant temporal frequency of 0.5 Hz was used with a variable bar width (spatial frequency). During recordings the animals were quiescent under general anaesthesia (sodium pentobarbitol) and a contact lens protected the optical quality of the cornea. It is clear that the optical quality of the eye of the very young kitten is inferior to that of the adult. But on the basis of ophthalmoscopic observation we feel that it is unlikely that optical factors limit the acuity determined for the younger animals. The eye was aligned to the screen by finding the optic disc ophthalmoscopically and projecting it on to the screen. Retinoscopy and trial lenses provided

an optical correction to the screen at 57 cm which covered a 40° visual angle The target had 80% contrast and a luminance of 24 cd m⁻²

For each size or spatial frequency of grating, 100 responses were recorded and averaged on a PAR Waveform Eductor to provide a good signal to noise ratio (Fig 1) Measurements were begun at 23 days of age, well after the hyaloid artery disappeared and the optical media were clear The amplitude of the evoked response was found to be a function of the spatial frequency of the pattern, confirming Berkley and Watkins[8] Visual acuity was taken as the highest spatial frequency for which a clear electrical response was present Spatial frequency thresholds for each animal were plotted on a log–log scale as a function of age (Fig 2, solid curve)
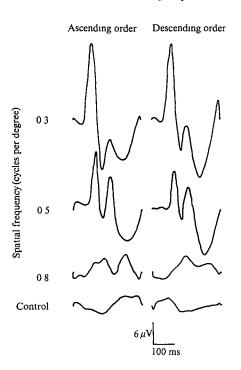
The points on the curve are, in principle, slightly above threshold because a clear though minimum response was used as the criterion At the next higher step of spatial frequency no response could be observed, so the exact threshold cannot be above these points which are represented by the dashed curve of Fig 2 This curve is at or slightly below threshold and is the limit of the maximum possible error in the minimum threshold as determined by this method

Grating resolution of the adult cats fell between 3 and 5 cycles per degree which is similar to that found by Berkley and Watkins[8] and by Campbell et al[9] using related evoked potential methods, and by Muir and Mitchell[10] using a standard psychophysical method Recently Blake et al[11] using conditioned suppression were able to approach 6 cycles per degree Wassle[12] found a comparable value for the optical upper spatial frequency cut-off using two parallel slits for the cat's eye, calculated at 4 to 5 arcmin or 6 cycles per degree Pure optical values would be expected to be the same or higher than the psychophysical acuity value of 3 to 5 cycles per degree These values seem low when compared with those of the human eye, the cat's optical system, however, is poorer[10] and its retina lacks a fovea centralis

It is clear from the trend of the curve that from 23 days of age, development of visual acuity in the cat is exceedingly rapid, and is virtually complete by 100 d At 23 d of age acuity was only 0 3 cycles per degree or 20/2000 Snellen Ten days later the acuity had improved to 1 cycle per degree or 20/600, an

Fig 1 Pattern evoked potentials from stimulation of the right eye of a 31-d-old kitten Measurements were recorded for different fineness of bar gratings (spatial frequencies) in ascending and then descending order The control was a static projection of the 0 3 cycles per degree pattern
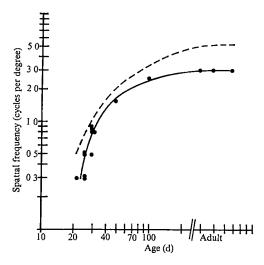
Fig. 2 The highest spatial frequency for which an evoked potential was present is graphed as a function of age (closed circles) A curve has been fitted by eye to these points (solid line) The dashed line joins the next higher spatial frequencies for which no pattern evoked potentials were present and is a measure of the maximum error Age scale for adult cats is in yr

enormous increase Between 33 d and 100 d the acuity approached the adult level of 3 cycles per degree, or 20/200

The optical media may become slightly clearer during the third to sixth week which could make a curve representing synaptic development slightly less steep Fig 2 may also be described as follows It " begins suddenly near the start of the fourth week, at about the time a kitten begins to use its eyes, and persists until some time between the sixth and eighth weeks, it then begins to decline, disappearing ultimately around the end of the third month " This is Hubel and Wiesel's description[2] of the sensitive period for changes in ocular dominance which fit our data Their descriptive data have been plotted by Blakemore[13] While other functions of the visual system may have somewhat different sensitive periods, it seems likely that all other functions fit within the limits of that described above for ocular dominance

Because the normal development of acuity takes place during the sensitive period, our findings support the hypothesis that this development may be used as a measure of the visual sensitive period A similar but non-invasive method (that is, using electrodes on the skin rather than on the brain) can be used to investigate the visual development of the human infant in order to study the human visual sensitive period and its time course

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Functional and neurochemical correlates of potentiation of striatal asymmetry by callosal section

AFTER lesions have been made in one side of the nigro-striatal system, rats have been found to rotate when administered drugs which release dopamine (such as amphetamines) or activate dopamine receptors (such as apomorphine) in the corpus striatum (see for example refs 1 and 2) Because this circling effect is specific to the nigro-striatal system[3] and because it has been established that rats consistently rotate contralateral to the more active striatum (for example ref 4), the lesion–rotation paradigm is now commonly used as a model for the study of nigro-striatal function Studies[5,6] in our laboratory have shown that rats without lesions also rotate when administered doses of the same drugs larger than those administered to lesioned rats As in lesioned rats, the direction of rotation is consistent for normal rats, when tested on several different occasions with the same dose of the same drug, some rats consistently rotate to the right and others rotate to the left This suggested that normal rats have an intrinsic asymmetry between left and right sides of the nigro-striatal system which is accentuated by drugs This functional asymmetry corresponds to a chemical asymmetry[7] the dopamine contents of normal left and right

Table 1 Effect of callosal section on rotation induced by (+)-amphetamine (1 0 mg kg⁻¹)

	Mean net rotations ± s e per 1 h	
Group	Preoperative	Postoperative
Callosal	48 2±14 6	97 7±12 2*
Sham	46 8±14 5	49 0±15 1

*Significantly different from preoperative value at $P < 0 01$, paired t test

striata were found to differ by 10–15% After administration of (+)-amphetamine, the dopamine contents of left and right striata were found to differ by 25% Moreover, in response to (+)-amphetamine, rats consistently rotated contralateral to the side containing the most dopamine This normal asymmetry suggests an inter-hemispheric mechanism that maintains or regulates the asymmetry Mensah and Deadwyler[8] demonstrated in rats a pathway between left and right caudate nuclei coursing through the ventral corpus callosum It seemed plausible that this pathway may be part of the mechanism maintaining striatal asymmetry We have now found that the striatal asymmetry is potentiated, both functionally and chemically, by callosal section

Twelve naive female Sprague–Dawley rats about 3 months old (approximately 250 g) were placed individually in a roto-meter modified[9] after that described by Ungerstedt and Arbuthnott[10] After 15 min each rat was injected intraperitoneally with (+)-amphetamine sulphate (1 0 mg kg⁻¹) Rotations were recorded automatically on a printout counter during the 15 min before and 60 min after injection Rotations to the left

or right during the pre- and post-injection periods were totalled separately and the net positive rotational difference (that is rotations in the dominant direction minus rotations in the opposite direction) was determined for each rat Approximately 3 h after testing, six rats were subjected to section of the anterior corpus callosum (from 0 5 mm posterior to bregma to the frontal pole) using procedures developed by Teitelbaum[11] Sham surgery was performed on the other six rats One week later, all rats were retested in the rotometer with the same dose of (+)-amphetamine All rats were then killed and perfused with 10% formalin, brains were removed and immersed in formalin for at least a week, after which sections (40 nm stained with Luxol blue and cresyl violet) were made and histological examination was conducted In all six rats of the experimental group, the anterior corpus callosum was severed as intended, except for slight cortical damage in one rat, there was no damage to other structures

Table 1 shows that whereas the sham-operated group made approximately the same number of rotations in both test sessions, the callosal-sectioned group made about twice as many rotations in the second session as in the first For all 12 rats, the direction of rotation was the same during both sessions Callosal section, therefore, only potentiated a normal bias

For the neurochemical experiment, six rats were again subjected to section of the anterior corpus callosum and six received sham surgery A week after surgery, each rat was killed by decapitation, its brain was removed and the left and right striata were dissected Each striatum was initially homo-genised by a Tissumizer in 4 0 ml of acetonitrile containing 0 2 μg of propionylcholine as an internal standard for analysis of acetylcholine The homogeniser was washed with an additional 3 0 ml of acetonitrile Homogenates were combined and centrifuged at 2,500 r p m for 10 min The acetonitrile phase was removed and assayed for acetylcholine by pyrolysis-gas chromatography[12] The pellet was resuspended in 5 0 ml of 0 4 N perchloric acid containing 0 1 ml of 10% EDTA, homogenised in a Teflon-glass homogeniser and then centrifuged at 15,000 r p m for 20 min in a refrigerated centrifuge Striatal dopamine was determined in the extract by a spectrofluorometric method[13]

Table 2 shows that callosal section had no effect on mean levels of striatal dopamine and acetylcholine The callosal section, however, enhanced a striatal dopamine asymmetry and induced a striatal acetylcholine asymmetry In the sham-operated group, there was a significant difference in dopamine content between striata, confirming previous work[7,14], and no significant difference in striatal acetylcholine content In the callosal-sectioned group, striatal dopamine asymmetry was approximately twice as great as in the sham-operated group, and an asymmetry in striatal acetylcholine content was significant Moreover, in the callosal-sectioned group, the striatal acetylcholine asymmetry was in the opposite direction to the striatal dopamine asymmetry, that is, for each rat, the side containing more dopamine had less acetylcholine In addition, for the latter group, the striatal dopamine asymmetry (H/L ratio) was inversely correlated with the striatal acetyl-

Table 2 Effects of callosal section on mean unilateral striatal dopamine and acetylcholine levels (μg g⁻¹)*

	Group	Left	Right	High	Low	Mean ratio (H/L)	
Striatal dopamine±s e	Callosal	6 87±0 51	6 57±0 49	7 67±0 44†	5 77±0 43	1 33±0 07‡	
	Sham	6 73±0 39	6 56±0 53	7 11±0 46†	6 18±0 38	1 15±0 04	
Striatal acetylcholine ±s e	Callosal	5 13±0 47	4 70±0 41	5 35±0 40†	4 48±0 41	1 19±0 05‡	
	Sham	4 82±0 32	4 65±0 23	4 94±0 34	4 53±0 25	1 08±0 04	

*Mean dopamine and acetylcholine were computed in two ways left side against right side, and side containing higher level against side containing lower level In addition, the mean high side/low side (H/L) ratio was also computed
†High side significantly different from low side (t tests, $P < 0 05$–$0 01$)
‡Callosal-sectioned significantly different from sham-operated (t test, $P < 0 05$)

Jline asymmetry ($r = -0\,92$, significant at $P < 0\,01$, t test), ere was no significant correlation for the comparable measurements in the sham-operated group ($r = 0\,21$)

/ These results support the initial hypothesis that a callosal pathway serves to regulate or maintain a set degree of balance or imbalance between the two striata After section of this pathway, the asymmetry is potentiated, both behaviourally and neurochemically The reciprocal relationship between the dopamine and acetylcholine asymmetries in the callosal-sectioned group is consistent with recent ideas concerning relationships between central cholinergic and dopaminergic mechanisms[15] If the normal asymmetry in striatal function is related to spatial preferences, as previous studies suggest[14,16], then different levels of activity in the intercaudate callosal pathway, as affected by reciprocal dopaminergic and cholinergic mechanisms, may be responsible for normal variations in sidedness among different animals

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Distribution of butanol molecules along bullfrog olfactory mucosa

ALTHOUGH electrophysiological[1] and gas chromatographic[2] studies have indicated that odorant molecules drawn into the intact frog olfactory sac establish a concentration gradient along the olfactory mucosa, conclusive evidence for such gradients requires a direct determination of how the molecules are actually distributed This requirement is met here by labelling butanol molecules with tritium and directly mapping their sorption along the mucosa

Bullfrogs (*Rana catesbeiana*) anaesthetised with urethane were mounted in a headholder and kept in a hood designed for use with radioactive gases The concentration (measured in partial pressure) of the tritiated butanol was controlled by a flow dilution olfactometer[1], from which the tritiated butanol was delivered to the frog's intact olfactory sac through a Teflon cannula placed in the external naris The stimulus was drawn into the sac as an artificially produced sniff of controlled flow rate This was accomplished using the negative pressure developed by a Harvard withdrawal pump which was connected to a Teflon cannula placed in the internal naris and secured there by a dental moulding material The stimulus
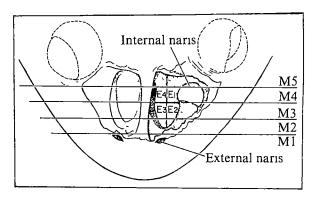
Fig 1 The relationship of the dorsal surface and eminentia sections to the intact olfactory sac of the bullfrog Immediately after the stimulus was presented, the frog was quick-frozen in liquid nitrogen This minimised any further movement of odorant molecules which could have resulted from diffusion and/or desorption With the animal frozen solid, a No 3 jeweller's saw was used to remove, in one piece, the dorsal, lateral, and septal walls of the olfactory sac To further section this piece and at the same time prevent its thawing, the piece was mounted in a specially constructed mitre box which could be maintained at a temperature below 0 °C In this way the dorsal surface could be sawed into five sections perpendicular to the long axis of the olfactory sac's surface These five sections were designated M1 to M5 with M1 being the section cut just caudal to the external naris and M4 being that part of the dorsal surface mucosa overhanging the internal naris When the dorsal, lateral, and septal walls of the olfactory sac were removed, its ventral surface (which contains the eminentia olfactoria) remained attached to the frozen carcass The eminentia was then cut into four sections designated E1 to E4 and each section was then removed from the animal All these sections (M1 to M5 and E1 to E4) were individually dissolved in tissue solubiliser and counted in a liquid scintillation system The number of disintegrations per minute for each mucosal section is an index of the total number of butanol molecules attracted to it The long black lines running the length of the figure refer to the dorsal surface, which is removed in this drawing E1 to E4 are indicated by the thicker crossed lines drawn along the surface of the eminentia

volume was controlled by the duration of the artificially produced sniff since, at a constant flow rate, duration determines volume Labelled molecules not adhering to the mucosal surface were collected in a toluene trap placed in the Teflon line between the internal naris and the withdrawal pump The animal was then frozen and sectioned (Fig 1)

The first column of Table 1 shows the distribution of butanol molecules resulting from the artificially produced sniff in which the partial pressure of the stimulus was 0 36 mmHg, the flow rate 16 ml min^{-1}, the duration 1 56 s, and the volume 0 42 ml

There is an obvious decreasing gradient of butanol molecules from the section which contains the external naris (M1) to that section overhanging the internal naris (M4), and the gradient is rather steep There is also a gradient, though less steep, rostro-caudally along the eminentia It is possible, however, that these gradients may simply reflect differences in the surface areas To correct for these differences, the percentage of the molecules recovered in each section was divided by the mean surface area for that particular section Correcting for surface area does not reduce these gradients (Table 1), indeed, the dorsal surface gradient is now even steeper

The comparatively small number of molecules sorbed by the eminentia compared with the dorsal surface is surprising This is perhaps caused by the poor location of the eminentia for the attraction of chemicals, which, like butanol, are heavily sorbed near the entrance to the olfactory sac, a region into which the eminentia does not even protrude Such observations raise questions concerning the role of different regions of the mucosa

If the gradient of odorant molecules along the mucosa has an effect on the processing of information by the olfactory system, the stability of these gradients as the stimulus intensity is varied becomes an important consideration[2] Therefore, holding volume and flow rate constant at the above levels, we compared

Table 1 Mucosal distribution of butanol molecules

Section	Total molecules recovered (%)	Mean surface area (mm²)*	Total molecules recovered (% mm⁻²)	Mean of all concentr % total molecules reco
Dorsal Surface				
M1 (most rostral)	84 38±1 29	4 5±0 1	18 75	84 59±1 98
M2	5 81±1 41	14 2±0 3	0 41	6 46±1 77
M3	1 86±0 85	19 0±0 5	0 10	1 60±0 89
M4	1 00±0 44	21 4±0 6	0 05	0 84±0 50
M5 (most caudal)	0 95±0 62	10 7±4 1	0 09	0 56±0 37
Eminentia				
E2 (rostrolateral)	2 85±0 75	3 7±0 2	0 77	2 37±0 91
E3 (rostromedial)	2 11±0 58	3 7±0 1	0 57	2 04±0 95
E1 (caudolateral)	0 42±0 21	4 3±0 2	0 10	0 33±0 19
E4 (caudomedial)	0 11±0 05	4 2±0 2	0 03	0 16±0 11
Not Sorbed	0 51±0 41	—	—	1 05±0 63
Number of animals	8	4		30

*Means based on four animals (comparable in weight to those used first two columns) which had not received any radioactive stimulus and were frozen and cut as described in the legend to Fig 1

Results (%) represent the number of molecules found in each section compared to the total number of molecules recovered Mean ± s d for that particular section taken from eight animals

the mucosal distribution patterns of butanol molecules established by five different partial pressures 0 06, 0 36, 0 62, 0 92, and 6 78 mmHg At least four animals were run at each partial pressure

Although the absolute number of butanol molecules recovered from each mucosal section increased with partial pressure, the relative number of molecules from section to section remained constant That is, an analysis of variance showed no significant differences among the percentages of the total number of molecules recovered from each section as a function of partial pressure Therefore, the results for each partial pressure are not presented individually, but are instead represented by mean values (last column, Table 1)

The fact that butanol establishes a steep concentration gradient and that this gradient remains unchanged throughout a wide range of concentration explains Mozell's earlier electro-physiological data[1] He showed that over a two log unit range of partial pressures butanol consistently produced measurable responses on the olfactory nerve branch supplying a region of the dorsal mucosa near the external naris, whereas the same butanol concentrations usually produced no measurable response on the nerve branch supplying the mucosal region overhanging the internal naris Mozell's interpretation of these data, namely, a rather steep mucosal concentration gradient, has now been directly and graphically confirmed by our radio-isotope study Only at the highest partial pressure obtainable at ambient temperature did Mozell observe significant activity on the internal naris nerve branch In terms of our study this would suggest that the saturated vapour, although still distributed along the mucosa in the same relative proportions, now provides a large enough absolute number of molecules at the M4 region to yield a measurable electrophysiological response

We do not mean to infer, however, that all odorants will have the same mucosal concentration gradient as butanol Indeed, from previous chromatographic[2] and electrophysiological[1] studies we predict that odorants with mucosal retention times[2] shorter than butanol, would, in a given sniff, have a less steep gradient Likewise, those odorants with long retention times might well show a gradient even steeper than that reported here

In summary, a butanol odorant stimulus results in the establishment of a concentration gradient along the intact olfactory sac and this gradient remains constant within a rather broad range of stimulus intensities This is what Mozell[2] predicted was necessary if a 'chromatographic-like' differentiation were one of the mechanisms basic to odorant discrimination Even if these distributions are not basic to olfactory discrimination themselves, they could be fundamental to any process which actually underlies olfactory discrimination For example, selectively tuned receptors along the flow path would be reached by different concentrations of the same odorant Therefore, this

initial distribution of the molecules along the olfactory receptor sheet is likely to be of central importance to our full understanding of both quality and intensity discrimination

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Triethylcholine as a precursor to a cholinergic false transmitter

A false transmitter is a substance that is stored and released by a nerve terminal instead of its normal transmitter Several false transmitters have been demonstrated at adrenergic synapses[1], but there has been no report of the synthesis, storage and release of a false transmitter at cholinergic synapses It has been suggested that the triethyl analogue of choline (2-hydroxyethyl-triethylammonium iodide, TEC) might form a cholinergic false transmitter, in vitro studies have shown that it can be accumulated by tissues that take up choline[2], and that choline acetyltransferase can acetylate TEC (refs 3 and 4) Nothing is known, however, about the specificity of the processes associated with storage and release of acetylcholine in intact tissue The possibility that TEC can form a false transmitter was studied in the present experiments The results demonstrate the uptake of TEC, its acetylation, and the release of acetyl-TEC by the superior cervical ganglion of the cat
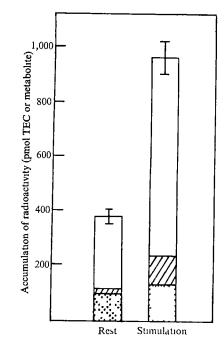
To measure the uptake and metabolism of TEC, ganglia were perfused[5,6] for 60 min with Krebs solution (mM NaCl, 120, KCl, 4 6, CaCl₂, 2 4, NaHCO₃, 25, KH₂PO₄, 1 2, MgSO₄ 7H₂O, 1 2, glucose, 9 9) containing ¹⁴C-TEC (*N*-ethyl labelled, 8 6 mCi mmol⁻¹, 10⁻⁵ M), in some experiments the preganglionic nerve was stimulated throughout (20 Hz, 0 3 ms, supramaximal voltage) Ganglia were removed, extracted with trichloroacetic acid (TCA, 10% w/v), and the TCA was removed with ether (initial experiments showed that TCA extracted almost all of the radioactivity accumulated by ganglia, <5% was extracted with phospholipids) The fate of TEC accumulated by ganglia was determined by shaking the aqueous

extract with tetraphenylboron in heptanone[7] (10 mg ml⁻¹), phosphoryl-TEC remained in the aqueous layer, but acetyl-TEC, and unchanged TEC were extracted into the organic phase from which they were recovered into hydrochloric acid (1 N, HCl) The HCl was removed by evaporation under reduced pressure, and the residue was incubated (30 °C for 30 min) with choline kinase (prepared from brewer's yeast, incubation mixture contained choline kinase 3 mg per ml protein, and (mM) ATP, 8 3, MgCl₂, 12 5, glycyl-glycine, 83, pH 8 5), TEC, but not acetyl-TEC, was converted to phosphoryl-TEC which was separated from the acetyl-TEC by extracting the latter into heptanone containing tetraphenylboron The breakdown of acetyl-TEC during these procedures was <5% In stimulated ganglia, 13 7±1 3% (mean±s e) of the accumulated radioactivity was phosphoryl-TEC, 10 6± 2 2% (102 pmol) was acetyl-TEC, and the rest was unchanged TEC The identity of these metabolites was confirmed by thin-layer chromatography In non-stimulated ganglia, total uptake of radioactivity was only 41% of that by stimulated ganglia (Fig 1), and less than 4% (<15 pmol) of the accumulated radioactivity was acetyl-TEC Thus, nerve stimulation increased the synthesis of acetyl-TEC, and also increased the uptake of TEC This extra TEC also accumulated in stimulated ganglia when synaptic transmission was blocked by tubocurarine (3×10⁻⁵ M) and therefore, it probably entered preganglionic terminals

In 13 experiments, ganglia were perfused first with ¹⁴C-TEC during preganglionic nerve stimulation (60 min), stimulation was then stopped, and perfusion was continued with TEC-free medium containing eserine sulphate (3×10⁻⁵ M), after washing out the radioactivity for 30 min, the preganglionic nerve was again stimulated to test the release of accumulated radioactivity In all experiments, nerve stimulation increased the release of radioactivity from ganglia (Fig 2a) In other experiments, the ganglion was not stimulated during exposure to TEC, little, if any, radioactivity was released from these ganglia during subsequent nerve stimulation (Fig 2b) This suggested that acetyl-TEC, not unchanged TEC, was released by nerve stimulation, and this was confirmed by treating the samples collected before and during nerve stimulation with choline kinase (see above) Radioactivity collected at rest was un-

Fig 1 Accumulation of radioactivity by cat superior cervical ganglia Ganglia were perfused (60 min) with Krebs solution containing ¹⁴C-TEC (10⁻⁵ M) at rest or during stimulation (20 Hz) of the preganglionic nerve Unshaded columns represent unchanged TEC pmol, mean±s e), hatched areas represent acetyl-TEC, stippled areas represent phosphoryl-TEC
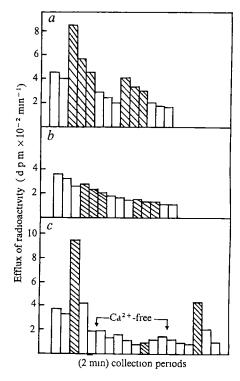
Fig 2 The effect of preganglionic nerve stimulation (20 Hz during collection periods indicated by the hatched columns) on the efflux of radioactivity from cat superior cervical ganglia perfused with Krebs solution containing eserine (3×10⁻⁵ M) The ganglia had previously been perfused for 60 min with Krebs solution containing ¹⁴C-TEC (10⁻⁵ M) during preganglionic nerve stimulation (a and c) or during rest (b), and then (all experiments) for 30 min (no stimulation) with TEC-free medium containing eserine In (c) perfusion was switched to a calcium-free medium (containing 10⁻⁴ M EGTA) during the time indicated by the arrows

changed TEC, and the extra radioactivity released during nerve stimulation was all acetyl-TEC The amount of acetyl-TEC released at the onset of the test period of stimulation was 19 1±2 9 pmol min⁻¹ (estimated from the extra radioactivity collected in the first 2 min of stimulation), this represents the release per minute of about 19% of the ganglionic content of acetyl-TEC, which suggests that acetyl-TEC is at least as available for release by nerve impulses as is acetylcholine under similar experimental conditions[8] The release of acetylcholine from nerve terminals is known to depend on the presence of calcium, and in two experiments, the release of acetyl-TEC by nerve stimulation was tested before, during and after perfusion with a calcium-free medium (containing 10⁻⁴ M EGTA) The release of acetyl-TEC was calcium-dependent (Fig 2c)

The present experiments show that TEC is taken up by cholinergic nerve terminals, that it is acetylated, and that acetyl-TEC can be released by nerve impulses in a manner similar to the release of the normal neurotransmitter Acetyl-TEC is pharmacologically inactive on cholinergic receptors[9], TEC, therefore, forms a false but inactive cholinergic transmitter

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Immunoreactive thyrotrophin releasing factor in gastropod circumoesophageal ganglia

REPORTS have indicated that thyrotrophin releasing factor (TRF) is stored in extrahypothalamic as well as hypothalamic brain regions of many vertebrates from the primitive larval lamprey to the more advanced mammals[1–4] Although TRF is found in the brain of many poikilotherms, administration of synthetic TRF (pGlu-His-ProNH₂) to these animals does not activate thyroid gland function[5–7] Thus, it has been proposed that, in these animals, TRF modulates synaptic transmission rather than releasing thyrotrophin (thyroid stimulating hormone)[1] Furthermore, the administration of TRF to hypophysectomised rodents potentiates the effect of the L-dopa on behaviour[8], thus supporting the hypothesis that TRF modulates monoaminergic transmission in higher vertebrates as well Further support for a role of TRF in synaptic transmission is the finding that administration of the synthetic tripeptide leads to an increase in noradrenaline turnover in rat brain[9–11] Because these reports imply first, that TRF may influence mono-aminergic transmission and second, that, in lower vertebrates, this role of TRF may be more important than that of regulating TSH release, we have investigated whether TRF is present in invertebrates which do not produce thyroid hormones but exhibit monoaminergic neurotransmission We found immunoreactive TRF in the circumoesophageal ganglia of various gastropods Species studied were the landsnail *Mesodon roemeri* (Connecticut Valley Biological Supply Co , Massachusetts), *Planorbis corneus* (Mogul Ed Corp , Wisconsin), *Helesoma trivolbis* and *Viviparus malleatus* (Ann Arbor Biological Center, Michigan)

The circumoesophageal ganglia were removed and freed of excess connective tissue under a dissecting microscope The ganglia were frozen immediately and tissues from several animals were pooled After thawing, they were homogenised in 100 μl of distilled water, and proteins were precipitated with 1 0 ml of absolute methanol After refrigeration overnight, the homogenate was centrifuged at 15,000g for 5 min and the protein concentration of the pellet was determined according to the technique of Lowry et al[12] The methanolic phase was dried under nitrogen and resuspended in phosphate buffer for radioimmunoassay of TRF The presence of immunoreactive TRF was determined by the method of Jackson and Reichlin[1] or by a modification of the method of Jeffcoate et al[13], to be described in detail elsewhere Both assays showed minimal crossreactivities with TRF breakdown products and structurally related small peptides The TRF determination in both assays, although carried out with different antibodies, were in good agreement (for example, 125 pg mg⁻¹ protein compared with 150 pg mg⁻¹ protein for a pool of ganglia)

Table 1 shows that TRF was present in various gastropods from the most primitive species examined (*V malleatus*) to the more advanced pulmonates (*H trivolbis, P corneus, M roemeri*) There was more TRF in circumoesophageal ganglia from the landsnail *Mesodon roemeri* than that in those from water snails (mean value of 308 pg mg⁻¹ protein compared with a pooled mean value of 44 5 pg mg⁻¹ protein) The amount of TRF in pooled snail ganglia was less than has been reported for hypothalamic tissue of various vertebrates[1], but was within the range of values reported for cerebral cortical tissues (20 pg mg⁻¹ protein in the rat to 4,500 pg mg⁻¹ protein in the tadpole[1]) Although not shown in Table 1, immunoreactive TRF was present in extracts from *Mesodon roemeri* after the methanolic extracts from the circumoesophageal ganglia had been purified on a column of Sephadex SP C-25 according to the method of McKelvy[13] This indicates that the immunoreactive material found in crude methanolic extracts from these animals is the tripeptide pGlu-His-ProNH₂ In further support of the identity of the immunoreactive material as TRF is the fact that the immunoreactive material from snail ganglia and synthetic TRF showed parallel dilution curves in the radioimmunoassay systems used

Thus immunoreactive TRF is present in various gastropod species This supports the evidence obtained from mammals that this molecule has a role in modulating neurotransmission, and suggests that this function evolved before that of controlling the release of thyroid stimulating hormone

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Table 1 Immunoreactive TRF in various gastropods

Gastropod species	pg TRF mg⁻¹ protein
Viviparus malleatus	31 5 (23–40)*
Helesoma trivolbis	65 0†
Planorbis corneus	37 0†
Mesodon roemeri	308 0 (75–720)‡

*Mean of two pools of ganglia, the range is indicated in parentheses
†Determination of a single pool of ganglia
‡Mean of four pools of ganglia, the range is indicated in parentheses

Possible placental origin of ACTH in normal human pregnancy

IT is well known that the free fraction of plasma cortisol is increased in pregnancy[1], but it has not been established whether this is a result of maternal pituitary or placental adrenocorticotrophic hormone (ACTH) secretion Maternal plasma ACTH levels in human pregnancy have been variously reported as elevated[2], or depressed[3], but there is no information on the relationship between ACTH levels and the stage of gestation We now report that maternal ACTH levels increase progressively throughout pregnancy, that urinary free cortisol levels are raised and show resistance to suppression by dexamethasone

and we present evidence suggesting that ACTH may be produced by the placenta

A total of 82 heparinised blood samples were collected between 0900 and 1100 from normal, pregnant women attending an antenatal clinic All samples were separated immediately at 4 °C and stored at −20 °C until assayed and all measurements were made within three weeks of sample collection Plasma ACTH was determined by radioimmunoassay after extraction on to porous glass[4] Highly purified human ACTH was used as standard and for iodination Three antisera were used, one directed against the N-terminal part of the biologically active amino acids 1–24 of the ACTH molecule (extended N-terminal antiserum), a second specific for amino acids 13–18 of the molecule (middle N-terminal antiserum), and the third against the biologically inactive C-terminus (C-terminal antiserum) The specificities of these antisera have been described in detail elswhere[4-6] Only the extended N-terminal antiserum was used to measure plasma ACTH, all three were used to measure ACTH in placental extracts
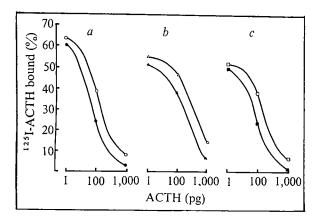
Fig. 1 Serial dilutions of a placental extract compared with dilutions of standard purified human ACTH using ^{125}I-ACTH, and N-terminal antiserum (*a*), and middle N-terminal antiserum (*b*), and C-terminal antiserum (*c*) ●, ▲, ■, standards, ○, △, □, placental extracts

Freshly collected placentas from normal deliveries were trimmed of cord and membranes and washed with 8 l of ice cold 4% saline Approximately 100 g of tissue was extracted in HCl-acetone (2 5 97 5 v/v)[7], ACTH was determined by radioimmunoassay in serial dilutions of these extracts In one extract, ACTH levels were also determined by cytochemical bioassay[8]

Excretion of free cortisol in the urine was measured by a competitive protein binding assay[9] The assay was modified for pregnancy urine by the addition of a preliminary wash with an equal volume of petroleum ether to remove progesterone which otherwise interferes in the assay This procedure removes 95% of ^{14}C-progesterone, but only 2% of the ^{3}H-cortisol[10] Determinations were made in 24 normal women, in 16 patients with pituitary-dependent Cushing's syndrome and in six women during the last trimester of normal pregnancy before and after the oral, administration of dexamethasone (2 mg d^{-1} for 3 d)

/ **Table 1** ACTH concentrations in human placentas measured by two radioimmunoassays

Extended N-terminal antiserum (ng per g wet weight)	C-terminal antiserum (ng per g wet weight)	Bioassay (ng per g wet weight)
3 2	2 8	0 66
11 3	16 7	
1 2	1 0	
21 3	13 1	
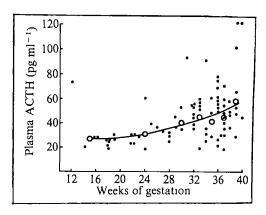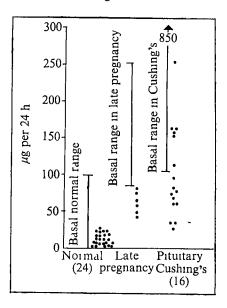
Fig 2 Maternal plasma ACTH levels in normal pregnancy Individual levels are shown (●) together with the mean levels (○) estimated after logarithmic transformation of grouped data (10–20 weeks, 21–27 weeks, 28–31 weeks, 32–33 weeks, 34–35 weeks, 36–37 weeks, 38–40 weeks) (Note the occurrence of a few very high levels which may have been caused by unrecognised stress during sample collection)

Plasma and placental extracts gave inhibition parallel to that of the standard (Fig 1) There was a progressive rise in plasma ACTH levels throughout pregnancy (Fig 2), although most lay within the normal range (<10–80 pg ml^{-1}) The placental content of ACTH ranged from 1 0 to 21 3 ng per g wet weight tissue (Table 1)

Following dexamethasone, normal subjects showed suppression of urinary free cortisol to less than 30 μg d^{-1} (Fig 3) Pregnant women, however, showed resistance to suppression, urinary free cortisol levels during dexamethasone administration were similar to those observed in some with pituitary-dependent Cushing's disease

These results agree with those of Genazzani and his colleagues[2], in showing elevated plasma ACTH levels during normal pregnancy, although, in contrast to these workers, the ACTH in maternal plasma shows parallel inhibition in our radioimmunoassay We have also shown a progressive rise in circulating ACTH concentrations throughout the second and third trimesters The discrepancy between these results and those of Mukherjee and Swyer[3], who found decreased levels, may be attributed to technical factors, since they did not use a plasma extraction step Other observations on maternal ACTH levels in human pregnancy cannot be strictly compared with

Fig 3 Urinary free cortisol levels before (vertical bars) and after (●) dexamethasone administration in normal women, women in the last trimester of pregnancy and patients with Cushing's disease

the findings discussed here because the samples were collected at the time of Caesarean section[11] or vaginal delivery[12,13]

The source of the increasing plasma ACTH levels may be the placenta or the maternal pituitary gland We have shown that urinary free cortisol levels in women in late pregnancy are resistant to dexamethasone suppression, a situation comparable with that observed in Cushing's disease This suggests that a component of maternal ACTH in pregnancy is not of pituitary origin The present studies, in agreement with those of Genazzani[2], and others[14-16] demonstrate high concentrations of ACTH in the human placenta Although content does not necessarily reflect either synthesis or release, these levels are comparable with those observed in tumours associated with ectopic ACTH secretion[17] and are much higher than could be accounted for by the content of sequestered blood This observation, together with the progressive increase in circulating levels during pregnancy and the elevated urinary free cortisol concentrations, suggests placental release of ACTH which is autonomous and not subject to feedback control

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Phosphorylase and glycerol production activated by cold in diapausing silkmoth pupae

THE accumulation of glycerol in overwintering insects, first shown in eggs of the silkworm, *Bombyx mori*[1], and pupae of certain saturniid silkmoths[2], is now known in many species Although not all cold-resistant insects contain high levels of glycerol, and the mechanisms of tolerance to cold are not fully understood, it is accepted that naturally produced glycerol can act as an antifreeze and contribute to protection against cold injury[3-5]

In certain insects, such as adult carpenter ants (*Camponotus pennsylvanicus*[6]) and Alaskan carabid beetles (*Pterostichus brevicornis*[5]), production of glycerol depends on exposure to cold In diapausing pupae of the silkmoth (*Hyalophora cecropia*) some glycerol is produced at 25 °C, but the rate of production is greatly increased at 6 °C (ref 7) Table 1 shows that haemolymph glycerol reaches about 250 mM at 25 °C and 500–600 mM after 1 month at 4 °C Glycerol is formed chiefly at the expense of glycogen[7], and enzymes forming an appropriate pathway occur in insect tissue glycogen phosphorylase[9], the glycolytic pathway to dihydroxyacetone phosphate, α-glycerophosphate dehydrogenase[10,11] and α-glycerophosphatase[12,13] Accordingly, cold might be expected to activate one or more steps in this sequence We have now demonstrated this
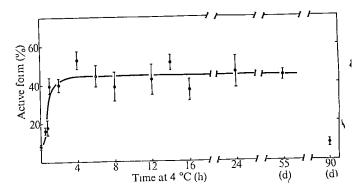
Fig. 1 Conversion of fat body phosphorylase to the active form after transfer of cecropia pupae from 25 °C to 4 °C Pieces of fat body were removed within 45 s and frozen between aluminium plates on dry ice The frozen tissue (about 100 mg) was homogenised in 1 3 ml of buffer (20 mM triethanolamine acetate, pH 7 0, containing 5 mM EDTA and 20 mM NaF) The homogenate was centrifuged twice at 10,000g for 10 min, and 25 or 50 μl of the supernatant was used for enzyme assay A coupled spectrophotometric assay[15] was performed in a Gilford 2400S spectrophotometer A rate was established in the absence of 5′-AMP, for active phosphorylase, then 2 mM 5′-AMP was added and a new rate was determined for total phosphorylase Values are means ±s e m from 3–10 determinations on individual pupae The initial level of about 8% active form is higher than that of less than 1% previously found by a different assay method[9], and recent experiments (L T Wimer, R Z and G R W, unpublished), show a systematic difference between the two assays, the reason for which is not known

We found in the fat body, which is the site of the major glycogen reserves in diapausing cecropia silkmoth pupae, activation of glycogen phosphorylase by cold that seems to play a role in the control of glycerol formation Phosphorylase in this tissue occurs in active and inactive forms, the latter (like muscle phosphorylase b) being activated by 5′-adenosine monophosphate[9,14] In fat body excised quickly from pupae stored at 25 °C, usually less than 10% of the phosphorylase was in the active form, but when pupae were placed at 4 °C, the active fraction increased rapidly to about 45% (Fig 1) After 3 months of storage at 4 °C, this activation had been reversed Similar effects of temperature were observed with fat body tissue *in vitro* (Fig 2) When pupal fat body was placed in Ringer solution, phosphorylase was converted partially to the active form

Table 1 Increase in glycerol content in haemolymph of cecropia silkmoth pupae after storage at 4 °C

Age of pupae (d)	Unchilled (25 °C) Glycerol (mM)	Chilled Days at 4 °C	Chilled Glycerol (mM)
30	147±9 (5)	5 5	171±13 (5)
70	278±35 (3)	30	490±80 (3)
90	225±54 (3)	40	679±57 (3)
110		50	512±50 (4)
110	205±27 (3)	60	540±46 (3)
110		70	438±45 (3)

Haemolymph samples were deproteinised with ethanol, and glycerol was determined with chromotropic acid[8] Means and standard errors, with numbers of samples in parentheses, are shown
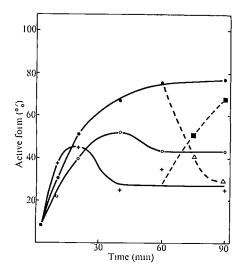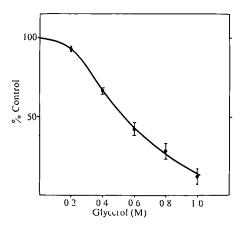
Fig 2 Changes in degree of activation of phosphorylase in fat body after incubation of the tissue in Ringer solution at different temperatures Fat body was removed quickly from diapausing pupae, rinsed very gently and divided into small pieces (about 100 mg), and each was placed in a small beaker with 2 ml of lepidopteran Ringer solution[16] at the temperature shown At the times indicated, the tissue was frozen between aluminium plates on dry ice Phosphorylase was then prepared and assayed as described in Fig 1 ●, 0 °C, ■ 0 °C after transfer; O, 15 °C; +, 25 °C; △, 25 °C after transfer.

(see ref 9), at 25 °C this activation was reversed after 20–40 min, but at 0 °C it continued, to produce up to 80% active enzyme at 90 min Incubation at 15 °C yielded an intermediate curve Transfer from 25 °C to 0 °C, or from 0 °C to 25 °C, after 60 min showed the effect of temperature to be reversible

Since the total level of phosphorylase did not change during these experiments, the activity changes probably reflected conversion of enzyme forms by phosphorylase kinase and phosphatase, respectively Both these activities have been demonstrated in soluble supernatant preparations under appropriate conditions, but in such preparations phosphorylase activation was not enhanced by cold The possible similar effects in other animal tissues, are being investigated

The association of changes in phosphorylase activity in cecropia silkmoth pupae with glycerol production is sup-

ported by our observation that the activation of phosphorylase in fat body at 0 °C is inhibited by addition of glycerol to the bathing Ringer solution (Fig 3) The level of glycerol giving 50% inhibition (about 0 5 M) is often attained in haemolymph *in vivo* This is suggestive of a regulatory feedback

These observations indicate that glycogenolysis in diapausing cecropia silkmoth pupae is controlled in part by the influence of both environmental temperature and the level of glycerol in the haemolymph on the phosphorylase system The specific conversion of carbohydrate to glycerol, however, is probably controlled at another step A regulatory role has been suggested[7] for α-glycerophosphatase and has recently received support[13] An effect of cold on this enzymatic step might account for the observation[17] that *Galleria* larvae contained elevated inorganic phosphate and decreased α-glycerophosphate after chilling at 0 °C

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Fig 3 Influence of glycerol on the activation of phosphorylase in fat body *in vitro* Pieces of fat body (about 100 mg) from a single diapausing pupa were incubated in 2 ml of Ringer containing different concentrations of glycerol at 0 °C After 60 min the fat body was treated and phosphorylase was assayed as described under Fig 1 The values are expressed as percentage of the increase shown by the control (60 min in Ringer with glycerol) Each value is the mean ± s e m of four determinations on different animals

Chromatographic demonstration of mineralocorticoid-specific receptors in rat kidney

THE binding of a drug with its specific macromolecular receptor(s) seems to constitute the first step whereby a number of pharmacological agents initiate a variety of intra and intercellular processes Aldosterone is the principal mineralocorticoid in mammals regulating ion transport in the kidney, and some extrarenal targets, but at higher concentrations it also exhibits glucocorticoid-specific properties[1] From studies on displaceable binding to protein ligands alone, it is not possible to affirm the physicochemical nature and homogeneity of a 'specific' mineralocorticoid receptor that is thought to be essential for the steroid function and said to exist in the toad bladder[2] and rat kidney[3] Techniques have been established for the characterisation and partial purification of corticosterone receptors in rat liver[4,5] These have now been adapted to demonstrate aldosterone-specific binding proteins present in rat kidney but absent from the liver

Figure 1 shows that aldosterone was bound to three sorts of proteins in rat kidney cytosol The first of these (I) was eluted with the low ionic (0 001 M) prewash on the DE-52 column and seemed to be the most abundant, the second (II) eluted at 0 006 M phosphate and was one-third as radioactive as the first, the third (III) component exhibited nearly the same concentration as the second but came off only at ionic strengths of 0 018 or more One or more of these could constitute the specific mineralocorticoid receptor(s) When a similar separation

was attempted with kidney cytosol equilibrated at 10^{-8} M corticosterone (Fig 2) there was only a faint indication of the first peak, the component in the 0 006 M PO_4 region was totally wanting, whereas nearly all of the radioactivity eluted between 0 02–0 06 M concentration of the phosphate From a series of other experiments, 5×10^{-9} M aldosterone was actually more effective than a high concentration (10^{-7} M) of corticosterone in revealing the components that peak in the 0 001 M and 0 006 M PO_4 regions (although 10^{-8} M aldosterone seemed to be required to saturate these macromolecules) In addition, the elution profile demonstrates that the first peak in the prewash does not represent free radioactivity or an artefact of the washing procedure[8] Although peaks I and II (Fig 1) may be explained as polymerised or aggregated forms of the mineralocorticoid receptor, this would be an unlikely postulate for peak III, in view of results shown in Figs 2 and 3

Mammalian kidney is endowed with gluconeogenic potential and is reported to possess macromolecules with high affinity for glucocorticoids[3] Peak III (Fig 1), which has the same elution position as the corticosterone peak in Fig 2, could therefore represent such receptors, or transcortin, or both When kidney cytosol equilibrated with 10^{-7} M ^{3}H-corticosterone was cochromatographed with ^{14}C-corticosterone bound to serum transcortin, a shoulder of radioactivity coming off at 0 018–0 02 M was masked by a much larger peak coeluting with serum transcortin at 0 06 M phosphate (Fig 3) Separation was sharper in the absence of serum and these peaks almost certainly represent the glucocorticoid receptors and transcortin, respectively, whereas peak III (Fig 1) and the peak in Fig 2 would seem to be a mixture of both of these components
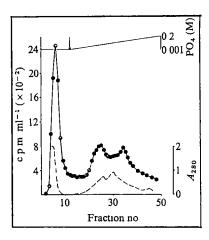
Fig. 1 Ion-exchange separation of aldosterone-binding proteins in rat kidney Male Wistar rats (150–200 g) were bilaterally adrenalectomised at least 48 h before use, exanguinated under light ether anaesthesia, and perfused with the initial buffer by aortic cannulation The excised organs were homogenised in an equal volume of the same buffer and the 105,000g supernatant fraction (6 ml) was equilibrated for 60 min at 4 °C with the desired concentration of labelled steroid (aldosterone, 10^{-8} M) The free radioactivity was then removed by additional incubation in presence of 100 mg ml^{-1} cell sap of activated charcoal (Sigma C-5260) for 10 min and centrifugation at 3,000g The supernatant was then passed through glass wool to remove traces of carbon and then charged on to the column The chromatography on DEAE-cellulose-52 (column 1×25 cm) was performed as described previously[4-6] The column was equilibrated with 0 001 M sodium phosphate, pH 7 5 Aliquots (1 ml) were mixed with 10 ml Unisolve (Koch Light) and counted in a Packard Tricarb Liquid Scintillation Spectrometer with corrections for quenching, spilling and background[7] After passage of 60–70 ml of the initial buffer (fraction volume 6–7 ml), protein was eluted (at arrow) with a linear gradient of 60 ml 0 001 M and 60 ml 0 2 M Na phosphate, at a flow rate of 60 ml h^{-1} (fraction volume approximately 3 ml) All manipulations were carried out at 4 °C 1,2,^{3}H-aldosterone (lot 1172, 55 Ci mmol^{-1}), 1,2,^{3}H-corticosterone (batch 57072, 26 Ci mM^{-1}) and 4,^{14}C-corticosterone (lot 467–248, 57 mCi mmol^{-1}), (Saclay, France) were diluted in ethanol, which was evaporated in the incubation vessel before equilibration All other chemicals were high purity reagent grade

Dotted line, A_{280} values, continuous line, radioactivity

Fig 2 Absence of corticosterone binding to mineralocorticoid specific receptors in rat kidney All details as in legend to Fig 1 except that the kidney cytosol (5 ml) was equilibrated with 10^{-8} M ^{3}H-corticosterone and loaded on the resin

Since mammalian liver is not the principal site of ion regulation, it is believed to be devoid of mineralocorticoid receptors[1,3] Figure 4 shows that the 0 006 M region had no radioactivity when liver cytosol equilibrated with 10^{-7} M ^{3}H-aldosterone was chromatographed under the conditions described above Competitive binding, followed by chromatography, further established that liver glucocorticoid receptors elute as a more abundant species in the 0 001 M prewash and a less abundant peak in the 0 018 region of the phosphate gradient (although the latter may also contain some residual, intracellular transcortin) and it is to these macromolecules that aldosterone seems to be bound (Fig 4)

These results thus provide chromatographic evidence that, as the principal ion regulator, the kidney is endowed with mineralocorticoid-specific receptors which are physicochemically distinct from the glucocorticoid binders, are present as readily distinguishable subpopulations within the cell and are wanting in the liver (having no active role in physiological ion regulation) Furthermore, they seem to be saturated at those endogenous, physiological concentrations at which binding of corticosterone

Fig. 3 Binding of corticosterone to glucocorticoid receptors and to transcortin Kidney cytosol (3 ml) and blood serum (2 ml) were saturated with 10^{-7} M ^{3}H-corticosterone and 0 25 µCi ^{14}C-corticosterone, respectively, and then treated with charcoal to remove free radioactivity After 60 ml prewash with 0 001 M phosphate buffer, elution was carried out (at arrow) with 60 ml each of 0 002 M and 0 2 M phosphate (fraction volume 4 ml) at a flow rate of 60 ml h^{-1} ●^{3}H, O, ^{14}C
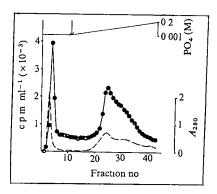
Fig 4 Absence of mineralocorticoid-specific receptors in rat liver All details as in legend to Fig 1 except that liver cytosol (5 ml) was equilibrated with 10^{-7} M ^{3}H-aldosterone and charged on the column

is still markedly dose dependent, and this is compatible with the fixed time assay value of KD_{37} °C 5×10^{-10} M (refs 1 and 3) Glucocorticoid specific receptors seem to be identical in the two organs, both with respect to the tenfold higher KD values and saturation with 10^{-7} M steroid, in keeping with levels circulating *in vivo*[1,3].

Purification of the receptor proteins is required to establish unequivocal affinity constants Nevertheless, the techniques described here may profitably be used to understand the mechanism of corticoid hormone action by analysis of receptor function in other organs, the stereospecificity of aldosterone binding in the presence of various agonists and antagonists of the steroid, possible qualitative differences in the relative abundance of the different subpopulations of steroid binders in various stages of differentiation, hypo or hyperfunction, and the relationship between the glucocorticoid and the mineralocorticoid receptors in diverse tissues

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Molecular structure of membrane-bound rhodopsin

WE have results which suggest a structure for the membrane-bound sensory protein, rhodopsin This structure has novel features which are of considerable biological interest

The transduction of light energy into neural signals is mediated in all known visual systems by a common type of visual pigment consisting of the 11-*cis* isomer of a retinaldehyde chromophore conjugated to some form of the protein opsin Photoisomerisation of a single molecule of the photopigment in a vertebrate photoreceptor cell can initiate a chain of events leading to excitation of that cell Although the primary events of the sequence are not known, it is widely suspected that they include a change in photopigment structure that causes release of some transmitter substance[1-3]

We looked for structure changes in rhodopsin-containing disk membranes using hydrogen–tritium exchange techniques[4,5]

Evidence for structure change has previously been limited to rhodopsin in detergent solutions[6-10] and some of these changes have been specifically shown not to occur *in situ*[10,11] Figure 1 shows that changes do occur in the membrane-bound protein following illumination The data in Fig 1 measure only the slowest 25 % of the exchanging protein hydrogens

To study the much larger fraction of protein hydrogens that exchange more rapidly, different exchange conditions must be used Figure 2 shows data obtained at lower pH where the intrinsic exchange rate of peptide hydrogens is 250 times slower Disk membranes from cattle and frog gave similar results While about 0 4 hydrogens per peptide group exchange rather slowly and trace out a series of slower and slower rates, a larger number, $\sim$ 0 7 hydrogens, form a clearly distinguishable and much faster kinetic class Surprisingly, the exchange rate of the fast phase is experimentally identical to that previously found for peptide group hydrogens that are freely exposed to bulk solvent water

The factors controlling the hydrogen exchange behaviour of free peptides have been calibrated in studies with small molecules[12] and homopolypeptides[13] Peptide exchange rates are a strong function of pH and temperature and vary to a minor extent depending on nearest neighbour side chains The calibrations obtained for these factors have been tested against hydrogen exchange data for the free peptides in a random chain heteropolypeptide, oxidised ribonuclease[12], in the globular protein, myoglobin[12,14], in the fibrous protein, collagen[15], and, by use of nuclear magnetic resonance techniques, for specific protons in the cyclic oligopeptide, angiotensin[16] In all these cases, the free peptide rates were well behaved and precisely predictable Under the conditions of Fig 2, free peptides are expected to have half times ranging generally from 30 to 60 s, and this is found for the fast phase in Fig 2

The possibility that protons from other sources might contribute significantly to these curves was considered in some detail The data in Fig 2 measure a total of 1 1 $\pm$ 0 1 exchanging hydrogens per peptide group This corresponds closely to the 1 12 $\pm$ 0 17 peptide and primary amide hydrogens expected on the basis of composition data for rod outer segment membranes[17-20] Moreover, experiments were carried out which excluded protons from phospholipids and protein side chains (These results will be detailed elsewhere) Thus the data in Figs 1 and 2 represent protein hydrogens

The data show that close to two-thirds of the peptide hydrogens in disk membranes are hydrogen bonded to solvent water Most of these must come from rhodopsin itself, since rhodopsin accounted for $\sim$ 85 % of the protein in our preparations, in agreement with the value established for uncontaminated rod outer segments[21-23] Thus we conclude that at least 60 % of rhodopsin's peptide hydrogens are freely exposed and hydrogen bonded to water This seems especially remarkable when it is considered that rhodopsin is an intrinsic membrane protein, and also that the more familiar water soluble proteins that have been studied by either hydrogen exchange or X-ray diffraction or both all have only 30 $\pm$ 10 % of their peptide groups hydrogen bonded to solvent water Some results for other membrane systems also indicate that their proteins, like typical soluble proteins, have only a small fraction of fast, freely exposed peptides (see ref 24 for sarcoplasmic reticulum and unpublished results of G Gabor and S W E for erythrocyte ghosts)

Rhodopsin's structure then must be quite unusual In addition to the large number of freely exposed peptide groups, a second restriction on possible structural models for rhodopsin is provided by its large complement of apolar residues Many of these are presumed to be in contact with the lipid hydrocarbon chains since the protein can be separated from the membrane only through the use of detergents The general, non-committal resolution of these apparently contradictory requirements seems to be that a good part of rhodopsin's polypeptide chain must be arranged at a lipid–water interface with many of its apolar side chains contacting lipid hydrocarbons and many of its peptide group protons in water

Several considerations argue against the surface of the membrane bilayer This surface could not provide the required apolar contacts, the highly polar phospholipid head groups are there Rhodopsin is thought to penetrate into and perhaps through the membrane, as suggested by fluorescence transfer experiments[25], by the pattern of protease sensitivity of the membrane-bound protein[26,27] and by freeze-fracture studies
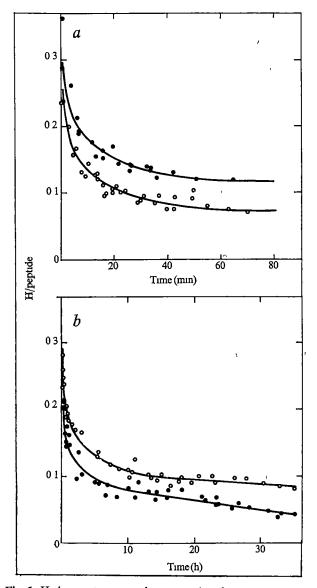
Fig 1 Hydrogen–tritium exchange-out data for disk membrane suspensions at pH 7 7, 0 °C The frog (a) and cattle (b) disk membrane preparations used had ratios A_{280}/A_{500} of 2 2–2 5 after purification on discontinuous sucrose gradients Exchangeable hydrogen sites were labelled to equilibrium with tritium in darkness by exchanging-in for 50–60 h at 0 °C in Ringer buffer with added tritiated water Exchange-out was initiated (zero time) by passing a sample of the suspension through a Sephadex column to remove the free tritiated water Subsequent separations allowed measurement of the amount of tritium still remaining bound as a function of exchange-out time, and the data were computed and plotted in terms of mol of original hydrogen as yet unexchanged per mol of peptide group present a, Disk membranes from frogs were either dark-adapted (●) or irradiated with white light at 0 °C for several minutes starting at zero time of the exchange-out experiment (○) When disk membranes from cattle were so treated (except that irradiation was with light of λ > 540 nm) no changes in exchange behaviour developed relative to the dark control b, Exchange-out of cattle disk membranes in the dark-adapted state (●) is compared with that of disk membranes containing opsin (○) Here the rhodopsin was converted to opsin by bleaching in white light and then incubating the disk membrane suspension for 45 min at 35 °C The suspensions were recooled to 0 °C before beginning exchange-out We do not know if the difference in response between cattle and frog disks represents a difference in the intermediates formed or in their kinetics of formation

of native photoreceptor membranes[28] Also against such a model is the consideration that this placement of the polypeptide chain seems to have little potential for function

A more compelling though still quite general possibility is that a good portion, perhaps 50%, of the polypeptide chain is arranged so that it forms the surface of an aqueous channel into the membrane In order to traverse a considerable fraction of the lipid bilayer and also contain enough bulk water to satisfy the requirement for hydrogen bonding to peptides at its surface, the channel would have to be of the order of 10–15 Å in diameter Analogous arrangements may be considered if two or more monomers cooperate to enclose the aqueous space[28]

The changes in hydrogen exchange behaviour found on illumination involve the slowly exchanging, internally bonded hydrogens corresponding to structured regions of polypeptide The free peptide region of the curve is unaffected by light (Fig 2) This matches the obvious expectation that the retinaldehyde chromophore is held within a structured part of the molecule, and is consistent with the supposition that this structure may act as a plug that closes off the rhodopsin channel in the dark but becomes distorted after illumination to
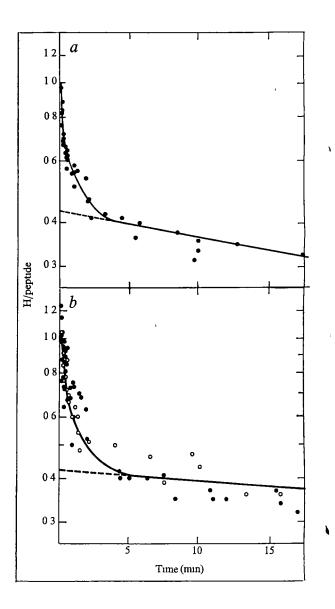
Fig 2 Exchange-out data for frog (a) and cattle (b) disk membranes at pH 5 3 and 0 °C Data from dark-adapted (●) and opsin-containing disks (○) are shown In both curves, 0 4 H/peptide group are slow and 0 7 form a faster kinetic class exchanging at just the free-peptide rate ●

allow the rapid escape of some transmitter substance contained within the disk membrane

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Photoreactivation of herpes simplex virus in human fibroblasts

When DNA is treated with ultraviolet light (220–300 nm) cyclobutyl pyrimidine dimers are produced between adjacent pyrimidines on a strand of DNA[1] These dimers are a major cause of mutations and death in simple organisms after ultraviolet-irradiation[1], and they have been implicated in the induction of skin cancer in man[2,3] An important tool for assessing the deleterious effects of dimers is the photoreactivation test the photoreactivating enzyme repairs DNA by the specific and exclusive monomerisation of dimers in a light-dependent (> 300 nm) reaction[4,5] If ultraviolet biological damage can be reversed by true photoenzymatic repair, then dimers have a major role in the production of that damage[6] For the test to assess the role of ultraviolet-induced dimers in human carcinogenesis at least three criteria must be met (1) human cells must possess a functional photoreactivating enzyme, (2) this enzyme must be able to monomerise dimers in DNA, and (3) the enzyme must be able to restore biological activity to ultraviolet-irradiated DNA It has been shown that human cells meet the first two criteria[7,8], we now show that human photoreactivating enzyme in fibroblasts can restore infectivity to ultraviolet-irradiated herpes simplex virus (HSV)

We exposed HSV in phosphate-buffered saline solution[9] to increasing doses of ultraviolet light from a low pressure mercury arc The ultraviolet-irradiated HSV was then titred on normal fibroblasts or those from patients with xeroderma pigmentosum, a rare genetic disease characterised by development of neoplasia in skin exposed to high levels of sunlight[3] It has been found that different cell lines derived from patients with
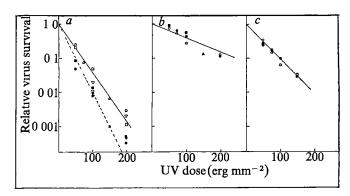
Fig 1 Photoreactivation of HSV in Jay Tim cells *a*, Jay Tim, *b*, HESM, *c*, XP-1, stocks of the F strain of HSV were grown and their titre was determined as before[9] Samples containing between 10^6 and 10^7 plaque forming units (PFU) of HSV in 1 ml of phosphate-buffered saline containing 0 1% glucose (PBS-glucose) were irradiated for various times with a 15-W GE germicidal lamp, ultraviolet dose was measured with a Jagger metre[13] calibrated with a Hewlett-Packard thermopile Immediately after irradiation the virus stocks were diluted with PBS-glucose and overlayed on fibroblasts in 25-cm² plastic tissue culture flasks at 37 °C Xeroderma pigmentosum cell lines were obtained from the American Type Culture Collection and normal human embryonic skin and muscle fibroblasts (HESM cells) were obtained from Flow Laboratories The growth and maintenance of these cells in Dulbecco's modified Eagle's MEM with 20% foetal calf serum has been described[8] Appropriate dilutions of virus were allowed to adsorb to the cells for 60 min in PBS-glucose and then the cells were overlaid with medium 199 containing 2% foetal calf serum and 0 4% human immune serum globulin The infected cells were either illuminated for 6 h with a 60-W yellow incandescent bulb at an average distance of 25 cm in an incubator at 37 °C or were kept in the dark by wrapping in aluminium foil Plaques were allowed to develop for 48 h and visualised by staining the infected cells with 2% crystal violet in 20% ethanol Virus survival was calculated from the relative plaque titres obtained by diluting unirradiated HSV and carrying out a parallel infection in illuminated or dark cells The open symbols represent titres on photoreactivated cells and the closed symbols represent titres on cells kept in the dark The different symbols represent different experiments The ultraviolet-dose curves on HESM cells were carried out in parallel with those on Jay Tim cells

xeroderma pigmentosum vary in both their levels of photoreactivating enzyme and the excision repair pathway[3,8] The ultraviolet-irradiated virus stocks were titrated on a xeroderma pigmentosum cell line lacking excision repair but possessing photoreactivating enzyme (Jay Tim) (Table 1) Infected cells were exposed to photoreactivating light for 6 h, duplicate cultures were kept in the dark and plaques were allowed to develop (Fig 1) Figure 1*a* demonstrates that virus exposed to 150 erg mm⁻² of ultraviolet light and plated in the dark on Jay Tim cells showed an apparent drop in titre by a factor of 1,000, in contrast, the titre of this virus was only reduced by a factor of 150 when cells were illuminated with photoreactivating light Since human photoreactivating enzyme has an action spectrum that extends beyond 600 nm (ref 16), we used a 60-W yellow incandescent light as our photoreactivating source to minimise damaging wavelengths in white incandescent light In the experiment shown in Fig 1*b* photoreactivation of HSV could not be detected when the virus was titred on normal human fibroblasts (HESM cells) containing both excision repair and photoreactivating enzyme (Table 2) The difficulties in detecting photoreactivation in excision-proficient cells have been discussed by Harm et al[10] The apparent drop in HSV titre at a given ultraviolet dose was considerably greater with xeroderma pigmentosum cells compared with normal fibroblasts This result is in agreement with previous reports on the effect of excision repair on ultraviolet-damaged HSV[11,12]

If the increased light-dependent survival of the HSV in Jay Tim cells is true photoenzymatic repair, cells which lack photoreactivating enzyme should show no effect of light on virus survival XP-1 cells contain neither appreciable excision

Table 1 DNA repair activity in normal and xeroderma fibroblasts

Cell line	Unscheduled synthesis (% of normal)	Photoreactivating enzyme activity (U)	Photoreactivating enzyme activity (% of normal)
Human embryonic skin and muscle	100*	627†	100
Jay Tim (XP12BE)	<2*	227†	36 3
XP-1 (XP1LO)	<2*	2 93	<1
Wo Mec (XP4BE)	100*	58 2	10 8

*See ref 3 for a complete discussion of unscheduled synthesis
†Photoreactivating enzyme activity was determined by the method of Sutherland and Chamberlin[14], protein was determined by the Lowry method[15] using bovine serum albumin as a standard Units of enzyme activity are pmol $mg^{-1} h^{-1}$

repair capacity nor photoreactivating enzyme activity (Table 1) HSV treated with 100 erg mm^{-2} showed a tenfold decrease in virus titre on XP-1 cells whether the cells were treated with photoreactivating light or kept in the dark (Fig 1c) In comparison, when cells were treated with white light from a 60-W bulb for 24 h after infection, the inactivated virus showed a 50-fold decrease in apparent titre. This was presumably the result of further damage to the infected cells by inactivating radiation present in the white light

We tested the contribution of photoprotection[13] to increased virus survival as follows Jay Tim cells were illuminated with photoreactivating light for 8 h before infection and then kept in the dark for plaque development We detected no appreciable difference in virus titre compared with unilluminated cells At a dose of 200 erg mm^{-2} we found the titre of the virus reduced more than 500-fold in both cases We therefore conclude that photoprotection does not contribute to the increased survival of HSV in Jay Tim cells treated with photoreactivating light We can also exclude any differences in the efficiency of plaque formation of HSV on xeroderma pigmentosum cell lines compared with normal human fibroblasts as a contributor to our observed results Several experiments with both types of cells kept in the dark yielded virus titres within 10% of each other when unirradiated HSV was used

Table 2 Effect of HSV infection on photoreactivity enzyme activity

Cells	Infected with 10 PFU per cell HSV*	Photoreactivating enzyme activity (% of line 1)
Human embryonic skin and muscle	No	100
Human embryonic skin and muscle	Yes	68 5
Wo Mec	No	10
Wo Mec	Yes	8 15

*Infection was carried out as described in Fig 1 Cells were overlaid with Medium 199 containing 2% foetal calf serum and harvested at 7 h after infection

These results show that the increased survival of HSV is caused by true photoreactivation It was considered possible, however, that such photoreactivation could be the result of the induction of an HSV specific enzyme after infection rather than to a human enzyme To exclude this possibility we assayed photoreactivating enzyme levels in HESM cells and in a xeroderma pigmentosum line (Wo Mec) with a low level of photoreactivating enzyme but normal levels of excision repair activity (Table 1) The data in Table 2 show that there is no increase in the photoreactivating enzyme level in HSV-infected HESM cells Thus virus infection does not increase photoreactivating activity *per se* In the Wo Mec cells there was no increase in enzyme activity after HSV infection (Table 2) This cell line should be an extremely sensitive indicator of appearance of any photoreactivating enzyme synthesised *de novo* provided there is normal expression of the HSV genome We have determined the yield of infectious virus from xeroderma pigmentosum

lines When the cells were kept in the dark we could obtain as much as 5–10 plaque forming units (PFU) of infectious virus per infected cell compared with a yield of 10–30 PFU per infected cells with HESM cells Such results, together with the efficiency of plaque formation of HSV on xeroderma pigmentosum cell lines, demonstrate that there is normal expression of HSV gene products during the infection of these cells We conclude, therefore that all the photoreactivation observed in the infected cells is caused by the presence of the human enzyme

We have demonstrated that the photoreactivating enzyme in human cells repairs UV-damaged DNA and restores its biological activity This demonstration confirms the biological significance of the human photoreactivating enzyme It is thus valid to use the photoreactivating test in evaluating the contribution of pyrimidine dimers in UV-induced human skin cancers

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Cyclic AMP and the production of sex pili by *E. coli* K-12 carrying derepressed sex factors

THE filamentous appendages, known as sex pili, determined by F-like and I-like sex factors in enterobacteria, have an essential function in conjugation[1] But neither the exact nature of their function, nor their mode of formation is yet known The number of sex pili ordinarily seen in cultures of *Escherichia coli* K-12 carrying derepressed F-like or I-like sex factors[2] (where pilus production is not limited by specific repression) seldom exceeds an average of 1–2 per bacterium With I-like pili, however, the numbers can be increased 50-fold or more by exposing the bacteria to either antiserum reacting with the pili[3], or vigorous washing[4]

Liquid cultures of strains of *E coli* K-12 producing sex pili due to a derepressed sex factor are commonly granular in appearance[5,6] Therefore, when very large clumps of bacteria visible to the naked eye were seen with a particular strain, M878, carrying the derepressed I-like sex factor, I*drd*16 (ref 7), this suggested that M878 was forming exceptionally large numbers of the sex pili determined by I*drd*16 The parent of strain M878 was PP78*cya crp*[8] (deficient in adenyl cyclase[8] and cyclic AMP receptor protein[9]), the tentative interpretation was,
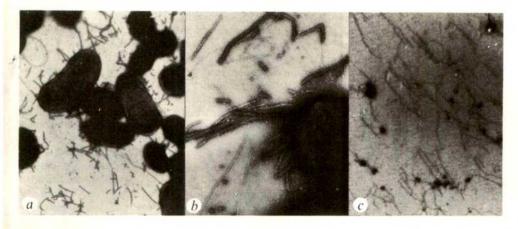
Fig. 1 *a, b*, Samples of a broth culture of strain 5336*cya crp*⁺ carrying R538I*drd*2, grown without cyclic AMP, showing numerous I-like sex pili, distinguished from F pili and common pili by attachment of specific antibody, as described by Lawn and Meynell[6]. Samples were fixed with 0.5% formalin before adding antiserum to avoid the possibility of an increase in pilus production caused by the antibody itself[3]. Comparison with the sample in *c*, which was treated with F pilus antiserum, shows that large numbers of I-like pili were indeed also present when they had not reacted with the antibody (*a*, ×7,700; *b*, ×23,760; *c*, ×16,280).
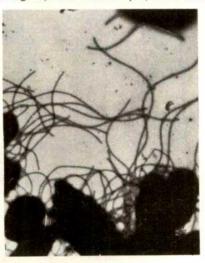
therefore, that the unusual behaviour of strain M878 was caused by the *cya crp* mutations. PP78 was derived from strain 1100 (*cya*⁺ *crp*⁺) (ref. 8) which, in turn, was derived from HfrH*thi*, strain 3000 (ref. 10). In addition to carrying I*drd*16, M878 had been made E1ʳ (resistant to colicin E1-K30) and carried ColE1-K30 (ref. 11). The *cya*⁺ *crp*⁺ control strain (M889) for M878 was, therefore, strain 1100 made E1ʳ and carrying ColE1-K30 and I*drd*16.

When cultures of M878*cya crp* were examined by electron microscopy, at least 100-fold more I-like sex pili were seen, mainly loose and scattered over the specimen grid, compared to cultures of the control strain, M889*cya*⁺ *crp*⁺. Since M878 and M889 both originated from HfrH, they were expected to form F pili, common pili and flagella, as well as I-like pili. However, only I-like pili were more numerous in M878 than M889. F pili and common pili were equally frequent in either strain but only a very rare flagellum was seen with M878. This confirms Yokota and Gots[12] who showed that cyclic AMP is required for flagella formation but that its absence does not decrease the numbers of F pili.

Similar results were obtained with two derepressed I-like R factors, R538I*drd*2 and R64*drd*11, after their transfer to strain PP78*cya crp* E1ˢ Col⁻.

To test whether increased formation of I-like sex pili resulted from the *cya* or the *crp* mutation, R538I*drd*2 and R64*drd*11 were transferred to the following strains: 5333*cya*⁺ *crp*⁹, 5336*cya*⁺ *crp*⁺ (ref. 8) (from strain 1100); CA8306*cya crp*⁺ (from HfrH, strain 3000); and WZ25*cya*⁺*crp* F⁻ (ref. 13). In each case, large numbers of I-like sex pili were formed. Thus the products of both the *cya* and the *crp* genes were involved in restraint of pilus formation and their effect was not confined to strain M878, originally tested.

Fig. 2 Sample from a broth culture of strain CA8306*cya crp*⁺ carrying R538I*drd*2, grown with 0.33 mM cyclic AMP. Large numbers of flagella, but no I-like sex pili, can be seen (×10,000).

The effect of the *cya* mutation on synthesis of catabolite-sensitive enzymes is reversed by adding cyclic AMP to the growth medium[8]. Strain 5336*cya* or strain CA8306*cya* carrying R538I*drd*2 were grown either with or without 0.33 mM cyclic AMP. The two strains behaved identically; without cyclic AMP, the cultures showed many I-like pili but only a rare flagellum (Fig. 1*a,b,c*); with cyclic AMP, they showed few I-like pili (considerably less than one per ten bacteria) but many more flagella than were observed in *cya*⁺ *crp*⁺ strains whether cyclic AMP was present or not (Fig. 2). Also, the bacterial bodies differed: with cyclic AMP, they had their usual bacillary shape, but without cyclic AMP, they were almost coccal. The numbers of F pili or common pili were not significantly affected.

In our experience, F is not typical of derepressed F-like sex factors inasmuch as it causes relatively few sex pili to be formed. The effect of cyclic AMP was, therefore, tested on four derepressed F-like R factors (two *o*ᶜ, R538F*drd*1 and R124*drd*2; and two *i*⁻, R1*drd*19 and R136*drd*M1: refs 14, 15) in strain 5336*cya crp*⁺. With all four R factors, about 10 times more F-like sex pili were present in cultures grown without cyclic AMP. The contrast was less striking than with I-like factors because more pili were formed when cyclic AMP was present.

We have, as yet, no clear indication of the level at which cyclic AMP acts in restraining sex pilus production. The effect of the *cya crp* mutations could be on pilus protein synthesis or its polymerisation into morphological pili. The fact that the cyclic AMP receptor protein is also required may indicate that, as with other prokaryotic functions where cyclic AMP is involved, cyclic AMP acts at the level of transcription[16]. It is notable that the effect of cyclic AMP and its receptor protein on pilus production is negative rather than positive. But the *cya* or *crp* mutations need not directly affect transcription of genes involved in pilus production, but may act indirectly through some more general effect on the bacterial cell envelope[17]. Some authors have suggested the possibility that pilus protein may be present as a constituent of the bacterial membrane when a sex factor is present (for example ref. 18). The bacteria of the *cya crp* strain, PP78, had an altered shape and, in conditions where they carried an I-like sex factor and produced large numbers of sex pili, the bacteria were lysed by low concentrations of sodium dodecyl sulphate (SDS) (~ 1%) which in the case of *cya*⁺ *crp*⁺ bacteria[6] only dissolved the sex pili without affecting the viability, microscopic appearance or motility of the bacteria. Neither did the same concentrations of SDS affect *cya crp* bacteria not carrying an I-like sex factor (K. Flint, unpublished).

Washing with broth or phosphate-buffered saline increases the number of sex pili[4] and removes intracellular cyclic AMP[19]. If the effect on pilus production caused by washing was a result of removal of cyclic AMP, it might be abolished by adding cyclic AMP to the wash medium. This could not be shown, however, despite repeated attempts. Samples of the strain, W945(R538 I*drd*2), with which the effect of washing was originally demonstrated, were washed with broth containing up to 3 mM cyclic AMP neutralised with NaOH, after growth in the same medium,

but the number of pili produced was similar to control preparations grown and washed with medium without cyclic AMP.

Even where a structural component has been identified in bacterial conjugation, as with the sex pili determined by F-like and I-like sex factors, a major difficulty in its study up to now has been its isolation in sufficient quantity. This difficulty should be overcome to a considerable extent by the greatly increased numbers of pili produced by *cya crp* mutant bacteria, as described here. Furthermore, several additional categories of sex factor exist for which a structural component corresponding to the sex pili of the F-like and I-like classes has not yet been demonstrated[20]. If such a structure were similarly produced in greater than normal amounts in *cya crp* mutant bacteria, the use of such bacteria may increase the likelihood of its identification.

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Synthesis of ribosomal RNA during sporulation in *Bacillus subtilis*

SPORULATION in *Bacillus subtilis* is associated with specific changes in RNA metabolism required for the expression of sporulation genes. Although net RNA synthesis stops abruptly at the end of logarithmic growth (T_0), active turnover and differential transcription of the genome have been demonstrated[1-3]. Losick and Sonnenshein[4] suggested that modifications of RNA polymerase may play a primary role in the regulation of gene expression during the sporulation process. The expression of ribosomal RNA genes during this period has been subject to conflicting reports[1,5-7]. The reported turn-off of these genes at the onset of sporulation[5,6] has not been observed in similar experiments[7]. The latter, and the reported changes in template specificity of RNA polymerase[4,8] were shown to be strongly dependent on the nature of the sporulation medium and to a lesser extent on the phenotype (*sp+* or *sp-*) of the strain[7,8]. We have analysed the pattern of synthesis and turnover of stable RNA components in *B. subtilis* induced to sporulate in the highly defined resuspension medium, SM (ref. 9). We report that rRNA synthesis does continue during sporulation, although at a reduced rate, resulting in the gradual replacement of vegetative rRNA components by similar newly synthesised sporulation species.

To label the stable RNA components during vegetative growth. *B. subtilis* strain *A26*, a uracil auxotroph, was cultured

in a synthetic medium containing ³H-uridine. These cells were centrifuged, resuspended in SM medium and samples were withdrawn at various times for a second labelling with ³²P-phosphate. Figure 1 illustrates the periods of labelling and some typical events connected with sporulation which were examined here, that is, nucleic acid synthesis, formation of alkaline phosphatase and heat-resistant cells. The appearance of these events are characteristic of cells sporulating in SM medium[9]. RNAs were extracted[14] in the presence of 2 mg ml⁻¹ bentonite[15] with 1% sodium lauryl sulphate added to protect the lysates from the new sporulation nucleases[16,17]. To evaluate the distribution of the labels among the RNA species and to eliminate the extraneous ³²P-labelled material known for its nonspecific binding to nitrocellulose membranes, the RNA preparations were first fractionated on methylated albumin-Kieselguhr (MAK) columns[18]. Figure 2 illustrates a typical MAK profile of an RNA sample taken from stage T_2 and cochromatographed with vegetative marker. The bulk of the ³H-labelled species chromatographed as stable RNA components with the expected 2 : 1 ratio of label in the 23S and 16S peaks, whereas the ³²P-labelled species had a ratio nearer to 1 : 1 indicating the presence of mRNA in addition to the stable RNAs. The profile also shows the efficient separation of the nonspecific ³²P-labelled material and low molecular weight species from the bulk RNA which eluted only after the application of the salt gradient. We calculated that in the RNAs from stages T_1, $T_{2.5}$, T_5, 15%, 30% and 60% of the ³H-label eluted as low molecular weight RNA, respectively. This change
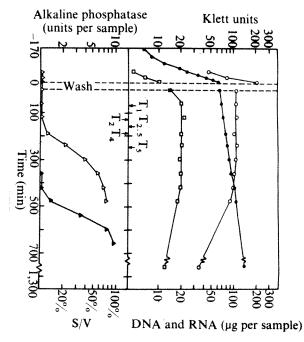
Fig. 1 Sporulation of *B. subtilis* in SM resuspension medium. A uracil auxotroph strain (*A26u⁻, sp+*) was grown in the presence of 5,6-³H-uridine (10 μCi ml⁻¹; specific activity 46.2 Ci mmol⁻¹, New England Nuclear) and 15 μg ml⁻¹ of unlabelled uridine. Turbidity (●) during growth was monitored by a Klett colorimeter using a 66 filter. At a Klett of 44, 100 μg ml⁻¹ of unlabelled uridine was added for a chase period of 20 min, the cells then were resuspended in SM medium containing 15 μg uridine ml⁻¹ for 20 h. Throughout the experiment, duplicate samples (9.5 ml) were removed and precipitated with cold TCA (final concentration 5%) and fractionated according to the Schmidt-Thannhauser procedure[10]. RNA (□) was determined by the orcinol reaction[11]; and DNA (○) by the diphenylamine reaction[12]. For the estimation of alkaline phosphatase activity (△), 5 ml samples were removed and treated according to Torriani[13]. Spores were estimated by viable counts before (V) and after heating (S) for 10 min at 80 °C and is expressed as sporulation frequency (▲, S/V × 100). At the indicated times after resuspension (T_1, T_2, $T_{2.5}$, T_4, and T_5), 200 ml samples were removed and labelled with ³²P-phosphate (33-70 μCi ml⁻¹) for 30 min followed by rapid chilling with an equal volume of frozen media.

Table 1 Hybridisation-competition behaviour of vegetative rRNA of *B subtilis*

Expt no	DNA Preparation no	Strand	DNA/RNA input ratio	Unlabelled rRNA competitor*	Complexed after RNase (c p m)†	³H-RNA hybridised (%)	DNA (H) or (L) hybridised (%)
1	I	H	50 1	—	1,650	36 6	0 73
		H	50 1	50 × (V)	18	0 4	
2	I	H	75 1	—	2,215	49 1	0 67
		H	75 1	50 × (V)	45	1 0	
3	II	H	200 1	—	4,490	99 7	0 50
		H	200 1	100 × (V)	43	1 0	
		H	200 1	100 × (S)	167	3 7	
4	II	L	95 1	—	2	0 04	0 0005

* Unlabelled messenger-free rRNA from vegetative (V) cells was extracted after treatment with rifampicin (30 µg ml⁻¹) for 20 min Similarly, unlabelled RNA from sporulating (S) cells at stage $T_{2\,5}$ was extracted after treatment with rifampicin (100 µg ml⁻¹) for 40 min

† Each reaction mixture contained 0 17 µg ³H-rRNA (specific activity, 26,500 c p m µg⁻¹) MAK-fractionated H or L strands, 6 × SSC in a total volume of 1 60, 1 28, 0 68 and 3 0 ml for experiments 1, 2, 3 and 4, respectively The reactions were incubated at 67 °C for 18 h and treated as described previously[18,19]

represented a turnover rate during sporulation of 12% h⁻¹ Similar calculations were not carried out with the ³²P-labelled RNAs because of the high content of extraneous phosphate

We have previously shown that in *B subtilis* 16 and 23S rRNA, all soluble RNA (4 plus 5S) and 80-90% of pulse-labelled RNA synthesised by vegetative cells hybridise to the H strand of DNA[18,19] Ribosomal cistrons account for only a small portion of the *B subtilis* genome[20] but their transcription products constitute more than 95% of the total cellular contents[20] The use of MAK-fractionated L and H strands of *B subtilis* DNA enabled us to achieve nearly quantitative hybridisation of stable and pulse-labelled RNAs (refs 18 and 19) The hybridisation properties of MAK-fractionated H strands to vegetative ³H-labelled rRNA are summarised in Table 1 The results shown confirm the following earlier observations none of the rRNA hybridised to the L strand, maximum hybridisation (99 7% input) was achieved at DNA/RNA ratios
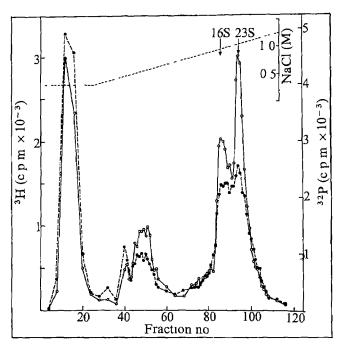
of 200 1, 0 25-0 37% *B subtilis* DNA hybridised with rRNA, addition of excess (50-100×) unlabelled messenger-free rRNA from vegetative or sporulating cells competed entirely with the available DNA sites The proportion of rRNA transcripts in the radioactive RNAs was determined by similar competition assays using the same H-strand preparations and the same unlabelled rRNAs as competitors The results in Table 2 are presented as a comparison between two pulse-labelled log-phase RNAs (experiments 1 and 2) and two independent sets of double labelled sporulation RNAs (experiments 3 and 4) Hybridisation competition of the double-labelled RNAs showed that 97-98% of the vegetative ³H-RNA that hybridised to the H strand was rRNA, whereas the ³²P-labelled sporulation RNAs were composed of both ribosomal and messenger species The proportion of rRNA in the various ³²P-labelled RNAs was characteristic to the sporulation stage and related to the overall rate of RNA synthesis One hour

Table 2 Hybridisation-competition behaviour of RNA preparations labelled with ³H-uridine during vegetative growth and with ³²P-phosphate during various sporulation stages of *Bacillus subtilis*

Expt no	Growth stage of pulse-labelling*	DNA/RNA input ratio†	Unlabelled competitor rRNA‡	Complexed after RNase (c p m) ³H	³²P	RNA hybridised (%) ³H	³²P	rRNA in labelled preparation (%) ³H	³²P
1	Log (3)	200 1	—	2,497	—	75 4	—	—	—
		200 1	100× (V)	1,422	—	43 0	—	57 0	—
		200 1	100× (S)	1,425	—	43 0	—	57 0	—
2	Log (8)	200 1	—	1,588	—	98 0	—	—	—
		200 1	100× (V)	599	—	35 2	—	64 1	—
		200 1	100× (S)	524	—	33 0	—	66 4	—
3	T_1	75 1	—	20,887	1,190	99 9	87 0	—	—
		75 1	50× (V)	565	856	2 8	62 5	97 2	28 2
	$T_{2\,5}$	75 1	—	5,360	890	99 9	55 2	—	—
		75 1	50× (V)	84	325	1 8	20 2	98 2	63 4
	$T_{5\,0}$	75 1	—	8,441	637	70 2	42 1	—	—
		75 1	50× (V)	109	183	0 9	12 1	98 3	72 3
4	$T_{2\,0}$	200 1	—	2,546	1,006	99 9	93 1	—	—
		200 1	100× (V)	56	337	2 2	31 2	97 8	66 5
		200 1	100× (S)	43	341	1 7	31 5	98 3	66 2
	$T_{4\,0}$	200 1	—	2,497	1,742	98 7	68 2	—	—
		200 1	100× (V)	50	407	2 2	15 9	97 8	76 7
		200 1	100× (S)	40	377	1 9	14 8	98 1	78 3

* Log (3) and Log (8) are log-phase cells grown in minimal salts[28] or in SM growth medium and pulse-labelled with ³H-uridine (44 or 17 µCi ml⁻¹) for a period of 3 and 8 min, respectively In experiments 3 and 4 the same amount of ³H-uridine (10 µCi ml⁻¹) was used, and the amount of ³²P-phosphate as µCi ml⁻¹ was 33 for T_1, $T_{2\,5}$ and T_5, 65 for T_2 and 70 for T_4

† The same H strand preparations described in Table 1 were used here, preparation I in experiment 3 and preparation II in 1, 2, and 4,

‡ The various reaction mixtures contained the following amounts of RNA in µg experiments 1 and 2, 0 06 and 0 35 (specific activities (c p m µg⁻¹), 55, 200 and 4,630, respectively), experiment 3, 2 87 (specific activity (c p m µg⁻¹) of ³H and ³²P, 7, 270 and 476) 0 83 (6,500 and 1,955) and 1 68 (7,160 and 900) for T_1, $T_{2\,5}$ and T_5, respectively), experiment 4, 0 5 (5,130 and 2,162) 0 5 (4,400 and 5 110) for T_2 and T_4 respectively The reaction mixture contained the appropriate amounts of MAK-fractionated H strand, 6×SSC in a total volume of 0 68 for experiments 1, 2, and 4 and 3 0 ml for experiment 3 The reactions were incubated at 67 °C for 18 h and treated as described previously[18,19]

Fig. 2 Chromatography of double-labelled RNA ^3H (O), ^{32}P (●) from sporulation stage $T_{2\,0}$ on a MAK column Isolated labelled RNA (0 1 mg, specific activity as c p m µg^{-1} of 5,130 and 2,160 for ^3H and ^{32}P, respectively) mixed with unlabelled marker RNA (1 06 mg) from vegetative cells, was applied to a MAK column (1 9 × 8 0 cm) and eluted using a linear salt gradient between 0 3 and 1 4 M NaCl in 50mM sodium phosphate, pH 6 7 (total volume, 600 ml) Fractions (4 ml) were collected and 1 0 ml samples were mixed with 3 0 ml scintillation mixture[27] The arrows indicate the peak absorbance (A_{260}) of the unlabelled 16S and 23S marker RNA Recovery of the unlabelled RNA (% input) total, 90 7, peak 1 (fractions 1–24), 9 2, 4S + 5S RNA (fractions 36–60), 14 3, and 16S + 23S RNA (fractions 72–105), 67 2

after resuspension in the sporulation medium (T_1), only 28 % of the ^{32}P-labelled RNA that hybridised to the H strand contained rRNA sequences, whereas at later stages, $T_{2-2\,5}$ and T_{4-5}, 66% and 78% of the ^{32}P label consisted of rRNA The latter values are comparable to the fraction of rRNA measured in pulse-label RNAs from vegetative bacteria (see experiments 1 and 2, Table 2)

We interpret the initial reduction in the labelling of rRNA at stage T_1 as being comparable to the temporary retardation of RNA synthesis and accumulation observed during a carbon-source step-down or amino acid deprivation of both relaxed and stringent bacteria[22,23] The SM resuspension medium[9] used here represents such a typical shift-down condition, which resulted in the characteristic decrease in the rate of RNA synthesis and more significantly in an RNA preparation that exhibited a smaller proportion of the label in rRNA As soon as the cells adjusted to the resuspension medium, the rate of RNA synthesis, particularly that of rRNA, increased When the pattern of RNA synthesis is studied in two commonly used sporulation media, the modified Schaeffer medium[24,25], or the Sonenshein and Roscoe medium[26] containing high levels of amino acids and glucose, this initial decrease in the rate of RNA synthesis was not observed[3] Instead, incorporation of radio-active precursors into RNA during 1-min pulse periods revealed a reproducible pattern of three periods of high rates of RNA synthesis at stages 0 to I, III and IV, the latter two were also observed in the SM medium used here (ref 3 and D T and R R, unpublished) We conclude that during the periods of increased RNA synthesis in sporulating *B subtilis* a substantial portion (65–80%) of the transcripts are copies of stable RNA genes The initial decrease in the rate and extent of rRNA synthesis which occurs only in certain media is not unique to sporulation, but rather represents the response of cells to growth rate transitions or to their entry into stationary phase This response involves the preferential restriction of rRNA transcrip-

tion until the cells become committed to sporulation or simply cease growing and remain stationary until lysis ensues We are currently investigating the transcription of rRNA cistrons during stationary phase of asporageneous mutants of *B subtilis*

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Note added in proof Pero *et al*, (*Spore*, VI (in the press)) report that in *B subtilis* sporulating in 121B medium[26] only 10% of a 3-min pulse-labelled RNA (T_2) represented ribosomal RNA

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Mefloquine, a clinically useful quinolinemethanol antimalarial which does not significantly bind to DNA

We report that mefloquine (2,8-bis-(trifluoromethyl)-α-(2-piperidyl)-4-quinolinemethanol[1], see Fig 1) clinically useful against drug-resistant *Plasmodium falciparum* malaria (G M Trenholme, unpublished), does not significantly bind to calf thymus DNA This constitutes the first reported case of an active compound in the quinoline–acridine class of antimalarials which does not strongly bind to DNA by intercalation

Investigations of the binding of acridine derivatives to DNA have provided information both on DNA structure and on the manner in which the complex of the dye with DNA can affect physiological functions Lerma[2], for example, tested the intercalation theory, using quinacrine, and Hahn[3] has carried out detailed spectroscopic investigations on the interaction of this compound with DNA An entire group of antimalarial compounds has been termed the quinoline–acridine class by Pinder[4] These compounds consist of a planar aromatic ring system with a positively charged side chain attached, and are typified by the quinolines, chloroquine and quinine, and the acridine, quinacrine Considerable evidence has accumulated that these three antimalarial compounds form an intercalated complex with DNA in vitro and can inhibit bacterial DNA and RNA synthesis by formation of an in vivo complex with DNA (ref 5) This has lead to a theory that the antimalarial action of these compound results from such a complex in the malarial parasite[5] Some controversy over this theory has arisen[6-8] but alternative postulations for the mode of action of these compounds ar
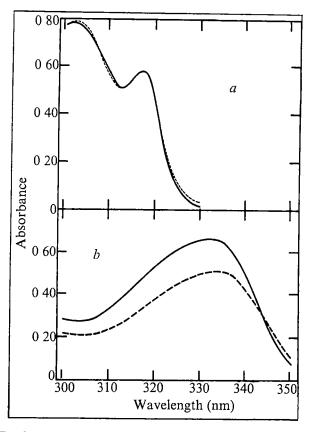
somewhat qualitative at present Recent synthetic efforts to find compounds which have good activity against chloroquine-resistant strains of malaria have resulted in a large number of compounds which can be used to study the postulated modes of drug action[9,10] As part of a continuing investigation of DNA interaction with antimalarials of the quinoline-acridine type, we initiated a DNA binding study of some new compounds which may be considered members of this class[11,12] This report extends our studies to include quinoline methanols

One method for determining whether an aromatic compound forms a complex with DNA is to follow shifts induced in its electronic absorption spectrum on the addition of DNA Figure 2 shows the ultraviolet spectra of mefloquine and quinine in the presence and absence of DNA The spectrum of quinine shows the familiar hypochromism and a shift of absorption maxima to longer wavelengths typical of compounds that bind to DNA, whereas that of mefloquine undergoes no significant change Thermal denaturation profiles conducted at a DNA phosphate and mefloquine concentration of 10^{-4} M changed DNA T_m by less than one degree The lack of significant change in T_m at this rather high ratio of compound to DNA phosphate also indicates at most a very weak interaction between mefloquine and DNA

Fig 1 Structure of mefloquine The sample used in these studies gave NMR, infrared, ultraviolet and melting point data in accord with published values

To quantify the mefloquine interaction with DNA, equilibrium dialysis studies were conducted using the buffer described in Fig 2, 2 × 10⁻⁴ M DNA phosphate, and mefloquine concentrations from 3 5 × 10⁻⁵ to 7 5 × 10⁻⁵ M (final concentration of unbound mefloquine after equilibration) The amount of mefloquine bound varied from approximately 3 5% to 6% of the total amount added and was too low for a Scatchard binding analysis Some estimation of the possible range of the binding constant is possible, however, if the number of mefloquine molecules bound per DNA phosphate is arbitrarily fixed For example, if one molecule binds for every DNA phosphate (electroneutrality) then the equilibrium constant can range from 400 to 700 If one molecule binds to every five nucleotides (mefloquine/DNA phosphate = 0 2), then the binding constant must be between 2,000 and 3,700 This is quite small compared with the range of 10⁵ to 10⁷ typically obtained for the binding equilibrium constant of drugs, the mode of action of which is known to involve binding to DNA A more detailed equilibrium dialysis study is impossible because of the limitations imposed on DNA concentration by the Donnan effect (at low ionic strength) and on the mefloquine concentration by the limitations of spectrophotometric determination of concentration

Almost any positively charged compound will become associated with DNA in a weak electrostatic complex as the ionic strength is reduced to low values To determine whether the weak binding observed with mefloquine is such an external electrostatic association or an intercalated complex, viscometric titrations have been carried out using methods described previously[12] The titration curves for two intercalating com-

Fig. 2 Effect of DNA on the absorption spectra of mefloquine and quinine *a*, Mefloquine hydrochloride 2 × 10⁻⁴ M (————), mefloquine hydrochloride 2 × 10⁻⁴ M plus DNA 10⁻³ M (—————), *b*, quinine hydrochloride 2 × 10⁻⁴ M (————), quinine hydrochloride 2 × 10⁻⁴ M plus DNA 10⁻³ M (—————) Solutions were prepared in 7 5 × 10⁻³ M NaH₂PO₄ and 10⁻³ M EDTA (standard buffer) adjusted to *p*H 7 with NaOH to yield an ionic strength of 0 019 Calf thymus DNA (Miles, Lot 36–155), which was used in spectrophotometric, viscometric, and thermal denaturation experiments, was dissolved in standard buffer and an extinction coefficient of 6,600/mol DNA phosphate (at 260 nm) used to calculate DNA concentration Drug stock solutions were prepared in standard buffer and diluted to the desired concentration before absorption measurements Difference spectra were recorded in 1-cm lightpath quartz cuvettes on a Beckman Acta V spectrophotometer We have found that mefloquine obeys Beer's law over a concentration range considerably larger than used in these studies, suggesting that aggregation of the drug is not a problem in these conditions and cannot account for the lack of intercalation with calf thymus DNA

pounds, a naphthothiophene methanol[12] and quinine, the structures of which are related to mefloquine, are shown in Fig 3 together with the result for mefloquine Both the compounds which intercalate cause a definite enhancement of DNA viscosity in contrast to mefloquine which causes a modest decrease in viscosity The latter result indicates that mefloquine does not intercalate, but could bind weakly to the DNA phosphate groups through an electrostatic interaction in a manner analogous to aliphatic amines and diamines[2,12]

The above results, taken together, strongly indicate that mefloquine does not intercalate with DNA, and when binding does occur at low ionic strength, it is by some weak external mechanism probably governed primarily by electrostatic interaction A compound with the same side chain and ring system(6,8-dichloro-2-phenyl-α-(2-piperidyl)-4-quinolinemethanol) without the trifluoromethyl moieties but with a 2-phenyl group, has been shown to bind strongly to DNA (ref 13) It is known that bulky groups, including trifluoromethyl groups, on aromatic ring systems can hinder intercalation[14,15] Model building experiments with CPK space-filling models indicate that a 2-phenyl substituent can lie in the same plane as the quinoline ring and that the entire aromatic system can intercalate between
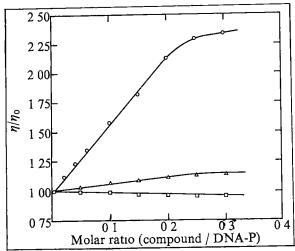
Fig 3 Viscometric titration of DNA with mefloquine hydrochloride (□), and quinine hydrochloride (△) The results for 6,8-dichloro-4-(2-N-piperidino-1-hydroxyethyl)naphtho[2,1-*b*] thiophene (○) were taken from reference 12, in which a more detailed procedure is given The ratio of the specific viscosity (η/η_0) of DNA with added compound (η) to DNA alone (η_0) is plotted as a function of the molar ratio of compound to DNA phosphate Viscosities were determined in a 1 2 dilution of the standard buffer with a Cannon-Ubbelohde four bulb dilution viscometer at 25 °C A measured volume of DNA $(2 \times 10^{-4}\text{M})$ was successively titrated with drug stock solutions in a calibrated (μl) syringe, and viscosities were corrected for slight changes in DNA concentration during titration

adjacent DNA pairs Similar experiments with mefloquine, however, suggest that the two trifluoromethyl groups introduce unfavourable steric interactions with DNA bases in an intercalative-type complex Since the binding constants of the smaller quinoline compounds, such as chloroquine[16], are lower than similar larger aromatic compounds, such as quinacrine[3], the unfavourable steric repulsion caused by the trifluoromethyl groups of mefloquine probably overrides the favourable hydrophobic interactions that would be obtained by intercalation Both model building studies and the experimental results described above are consistent with the conclusion that mefloquine cannot bind to DNA by intercalation

This work does not provide direct evidence for the mode of antimalarial action of mefloquine, however, it does indicate that DNA intercalation is not of primary importance and raises important questions about the mode of action of other unstudied compounds in the quinoline–acridine class of antimalarials Identification of cellular receptors other than DNA will be important in understanding the mode of action of mefloquine and perhaps other antimalarials of this class Since synthetic chemists have frequently assumed an intercalative DNA-binding mechanism of action similar to that of chloroquine to design potential antimalarials[17-20], the fact that mefloquine binds very weakly and does not intercalate with calf thymus DNA will be important in this field We are currently studying the interaction of several other quinoline methanol antimalarials with DNA to determine whether DNA binding could be an important part of their mode of action

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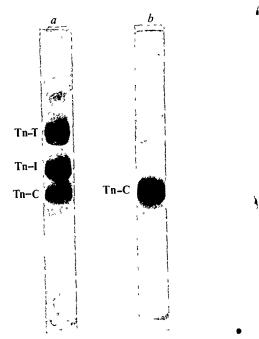
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Crystallisation of troponin-C

TROPONIN is the protein complex that controls the onset of muscular contraction[1-3] It has three subunits[4] troponin T (Tn-T), troponin I (Tn-I) and troponin C (Tn-C) The complex is bound at regular intervals along the thin filaments, which are composed of a double helix of actin monomers and tropomyosin, which runs along the grooves of the actin helix[5-7] Each tropomyosin molecule spans seven actin monomers and one molecule of the troponin complex is bound to each tropomyosin molecule through Tn-T (refs 3 and 6–8) Tn-I binds to actin and Tn-C binds both to Tn-T and Tn-I (refs 3, 4 and 6) In the absence of Ca, Tn-I and Tn-T inhibit contraction by inhibiting the interaction of actin and myosin but in the presence of Ca, Tn-C binds to Ca, the inhibition is reversed and contraction occurs[3] We have been interested in crystallising Tn-C in the hope that an eventual crystal structure determination will be helpful in understanding how Ca binding controls the onset of muscle contraction Here we report the occurrence of a crystalline form of this protein

Fig 1 SDS gel electrophoresis *a*, of rabbit troponin (35 μg) and *b*, Tn-C (35 μg) separated by column chromatography (in this example, using DEAE-Sephadex A-50)
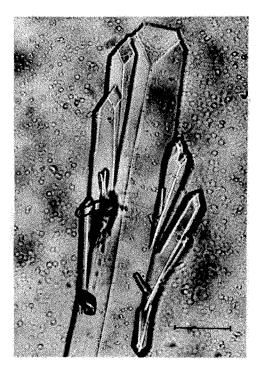
Fig. 2 Photomicrograph of crystals of pure rabbit Tn-C photographed in mother liquor with unpolarised, unfiltered light (×20). Bar represents 100 μm.

Rabbit troponin was prepared by the method of Ebashi *et al.*[9]. The three components were separated using column chromatography with either DEAE-Sephadex or A-50 (ref. 4) or Whatman cellulose DE-52 (ref. 6). Sodium dodecyl sulphate (SDS) gel electrophoresis[10] with heavy loadings of Tn-C invariably showed a single band with a molecular weight of 17–18,000 (Fig. 1) and all the Tn-C preparations, when combined with Tn-T and Tn-I, conferred Ca sensitivity on the ATPase of actomyosin.

For crystallisation a 25 mg ml^{-1} solution of protein in 50 mM Tris-HCl 5 mM $MnCl_2$, pH 7.0, was dialysed in Zeppenzauer cells[11] against 35% saturated ammonium sulphate, 5mM $MnCl_2$, 50 mM Na acetate, adjusted to pH 5.0 with concentrated acetic acid. All chemicals were BDH reagent or Analar grade; no other precautions were taken. For dialysis, ordinary Visking tubing was used that had been boiled for 5 min in 0.1% $NaHCO_3$. Crystals (Fig. 2) form within 4–8 weeks and are long bipyramidal tetragonal needles. They often show multiple growths roughly parallel to the long axis, sometimes rotated at either 45° or 90° about the long axis relative to the larger crystal.

Typically, crystals are 0.1 mm long. Occasionally large crystals (0.5 × 0.05 mm) appear and these have been used for preliminary X-ray diffraction studies[12]. The crystals give rise to three-dimensional diffraction patterns which confirms the existence of single crystals. Rotation of the crystals by 90° about the long axis gives rise to superimposable precession photographs. The patterns are weak, however, and generally limited to 8–10 Å resolution, occasionally to 3 Å. The observed reflections may be indexed on an 'I' lattice and there is evidence (G. Dodson, B. B., D. M., and J. P., unpublished) for a 4_1 or 4_3 screw axis parallel to the long dimension of the crystals. Thus at low resolution the crystals seem to be tetragonal with a body centred unit cell of 127 Å × 156 Å. More extensive data will be necessary, however, to confirm and extend these results.

The role of $MnCl_2$ in the crystallisation procedure is obscure. It is known that certain divalent cations such as Mg can compete for at least two Ca sites[12]. For these sites, the Ca-binding constant of troponin is about 5×10^8 mol^{-1} in the absence of Mg, but about 5×10^6 mol^{-1} in the presence of

2 mM Mg. In general, all divalent cations with a crystal radius of 0.8–1.1 Å that can displace Ca are also capable of activating the ATPase of myosin in the presence of troponin-containing thin filaments inhibited by ethyleneglycol-*bis*-(β-aminoethyl ether)N_1N'-tetraacetic acid[13]. Mn is in this size range (0.8 Å) and we have confirmed the result of Konieczny and Weber (unpublished, cited in ref. 13) that Mn is active in stimulating the ATPase to the same extent as Ca. Smaller ions such as Mg, Zn, Co, and Ni are inactive in stimulating the ATPase[13]. These results closely parallel the ability of different ions to induce conformational changes in Tn-C similar to those produced by Ca, as judged by ultraviolet spectroscopy[14].

The conformation and physical properties of Tn-C depend on the degree of saturation with Ca and these changes are evident in the properties of the troponin complex[3,4,6,14,15]. For example, Ca increases the affinity of Tn-C for Tn-I (refs 6 and 15). Similarly, Ca increases the affinity of Tn-C for Tn-T (ref. 6). It is likely that the Ca-dependent conformational changes in troponin Tn-C are involved in the control of the position of tropomyosin with respect to the actin helix[3,8,16,17]. In the presence of Ca, tropomyosin is moved to a position nearer the actin groove and actomyosin formation occurs. The Ca-dependent changes in Tn-C are likely to be the first steps in a series of structural events that constitute the control of muscular contraction. Thus a knowledge of the crystal structure is an important step in understanding this system.

We thank Professor S. V. Perry for a gift of rabbit Tn-C and Professors D. C. Phillips and J. W. S. Pringle for their encouragement.

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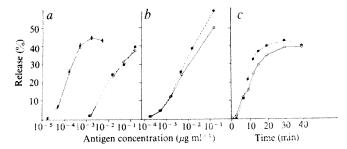
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Release of leukocyte kallikrein mediated by IgE

BRADYKININ is a vasoactive nonapeptide believed to mediate various acute inflammatory conditions[1]. Although its production in plasma is well defined[1], an understanding of its role in disease is hampered by the lack of information about the mechanism by which it can be generated by an immune process or by any stimulus other than clotting. Immune release of a kinin-generating factor from perfused sensitised guinea pig lung has been reported[2,3]. The release is not, however, calcium-dependent and the factor may be a product not of the primary allergic reaction, but of a secondary event[4]. Substantial information is available, however, about the release of other mediators of inflammatory or allergic reactions—histamine, SRS-A, and ECF-A[5]. These are secreted after antigen challenge of basophils

Fig. 1 The release of histamine and TAMe esterase activity from human leukocytes. *a*, Dose–response relationships of antigen-induced release. The precision of the esterase assay is indicated by the standard deviation shown in the release curve caused by rye grass group I antigen (left-hand curve). The other two frames represent *b*, release by highly specific anti-IgE; *c*, kinetics of the release of the esterase (●) and histamine (○).

and mast cells sensitised with IgE antibody[6-7]. The release depends on calcium and temperature, requires energy and is controlled by hormone-receptor interactions which influence the intracellular level of cyclic nucleotides[10-13]. We have now demonstrated the immune release of an enzyme from human leukocytes that hydrolyses *p*-toluene sulphonyl-L-arginine methyl ester (TAMe) and generates bradykinin from citrated human plasma. The release is initiated by the interaction of antigen or anti-IgE with cell-bound IgE[6,14] and seems to be similar in mechanism to the release of histamine and the other mediators of the allergic response.

Human leukocytes from donors allergic to ragweed or grass were separated from the other formed elements of the blood, washed free of serum and suspended in a serum-free Tris-buffered medium containing 0.03% human serum albumin, calcium and magnesium as previously described[10]. The immunological reaction was initiated by addition of antigen or anti-IgE to the cell preparations and the reaction proceeded for 30–45 min in the dose–response studies or for increasing periods in the kinetic studies. At the completion of the reaction, cells were centrifuged and the histamine released into the supernatant as well as that present in samples of untreated cells was determined spectrophotofluorometrically[10]. Arginine esterase activity of the supernate was determined radiochemically using ^{3}H-TAMe, a method which was devised by Beaven *et al.*[15] to measure human urinary kallikrein and modified for determination of prekallikrein[16] and arginine esterase activity in supernatants[17]. The total cellular arginine esterase activity was determined using sonicated samples of untreated cells. Bradykinin was generated by treating citrated human plasma with the supernatant from cells challenged with antigen or anti-IgE and measured by a radioimmunoassay with a sensitivity of 4.0 ng as previously described[18].

The dose–response relationships of the release of histamine and TAMe esterase have been studied with leukocyte preparations from more than 20 allergic individuals challenged with either the purified protein antigens from ragweed (AgE)[19] or grass (Gp I)[20] or with highly specific anti-IgE[21]. Typical dose–response curves are shown in Fig. 1. The precision of the assay for histamine is 5–10% while that for the TAMe esterase is about 3%, as shown in Fig. 1*a*. In general the pattern of the dose response curves for histamine and esterase release were similar, whether release was initiated by antigen (Fig. 1*a*) or anti-IgE (Fig. 1*b*). The absolute percentage of histamine or TAMe esterase released by a leukocyte preparation may differ.

Rates of histamine and esterase release are compared in Fig. 1*c*. The time courses were essentially identical: this was confirmed in eight experiments. Further experiments demonstrated that the reaction did not proceed in the absence of calcium ions or at 4 °C. Moreover, the addition of EDTA or lowering the reaction temperature immediately stopped the release of esterase at any point in the reaction, as described for histamine release[10].

The interaction of the TAMe esterase with the kinin-generating system was studied. Using kaolin as a positive control to generate prekallikrein activator from Hageman factor it was observed that the supernant from antigen-stimulated cells was inactive: no additional TAMe esterase activity was noted after its interaction with plasma. This suggests that the leukocyte enzyme does not function as or cause the production of any prekallikrein activators. It does, however, generate kinin activity from citrated human plasma. In four experiments 30–350 ng of bradykinin was produced after incubation of the esterase with plasma for 20 min; buffer incubation with the same plasma yielded less than 4 ng of bradykinin (the limit of the assay). In a typical experiment the esterase or buffer was incubated with plasma in duplicate and the kinin generated was assayed in quadruplicate. The buffer control was below the limits of detection (< 4 ng) in each case whereas the esterase-treated plasma had 33.4 ± 3.2 ng of bradykinin. Preliminary experiments have shown that the esterase can generate high levels of bradykinin from highly purified kininogen. It would appear, therefore, that the release of the TAMe esterase is associated with kallikrein activity. Whether the two activities are subserved by the same enzyme, however, remains to be ascertained.

The parallelism in dose–response and kinetics of kallikrein and histamine release, as well as the release by anti-IgE, indicates that the kallikrein is derived from human basophils: previous work has demonstrated that basophils constitute the only type of white blood cell that fixes IgE and contains histamine[22]. Phagocytosis by polymorphonuclear leukocytes, induced by latex beads or immune complexes, releases some materials with protease activity but these are not kallikreins[23]. This, therefore, seems to be the first time that the generation of kallikrein from human leukocytes has been observed as a result of a primary immune reaction, and represents an important link between reactions of immediate hypersensitivity and the plasma kinin generating system. The demonstration of the IgE-mediated release of a basophil kallikrein allows us to begin to study the role of this system in the pathogenesis of acute inflammatory responses.

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reviews

This book* must fascinate everyone interested in the history of British zoology in the 19th century. It begins with Joseph Banks still in the seat of power, his soirées at 32 Soho Square attended by eminent scientists discussing the latest 'curiosities'. Over his shoulder one has a glimpse of Hans Sloane whose collections, gradually disintegrating, were housed in subterranean excavations, said to resemble the catacombs of Palermo, in Montagu House. The greatest collections remained in possession of the landed gentry and nobility; even those offered to the Government were largely refused because of lack of space in which to keep them. Appointed by patronage, the miserably paid Keepers of Natural History had neither status nor influence.

The gradual changes which led to the organisation of the greatest collections in the world occupied the whole of the Victorian era and were the work of two keepers, John Edward Gray and Albert Gunther, their lives and work here admirably surveyed by the grandson of the latter. The major events are the erection behind Montagu House of the British Museum with the transfer there of the Natural History collections in 1840 and 1841 and the far greater move, in distance and bulk of material, from Bloomsbury to South Kensington in 1882 and 1883.

Born in 1800, the son of a struggling botanist and pharmacist, John Gray proceeded by devious occupations into medicine, to emerge, by virtue of character and ability, as a leading naturalist. Friendship with W. E. Leach, then too briefly at Montagu House, introduced him to Banks and his circle. His first Museum employment, at a daily payment of 15 shillings, was during the keepership of J. G. Children whom he succeeded in 1840. Initially, with his fellow keepers he was subject only to the Principal Librarian with direct access to the Trustees, this changing in 1856 when Richard Owen was appointed superintendant of all the natural history departments.

The spirit of Linnaeus, tempered now by that of Cuvier, prevailed and the great task presented to Gray was

*A Century of Zoology at the British Museum—Through the Lives of Two Keepers. By Albert E. Gunther. Pp. 533+37 photographs. (Dawsons: London, 1975.) £17.30.

The new Coral Room

Recollections of a century

the accumulation of specimens with "the diffusion of instruction and rational amusement". He travelled widely, visiting museums and meeting their directors and staff. Material began to pour in particularly from the colonies; collections of birds were purchased from John Gould, collections of shells from Hugh Cumming. Gradually, the collections came to rival in extent that of the still growing empire, although the Admiralty and Foreign Office were to disregard its claims until the conflict over the *Challenger* collections led to the final deposit of these in the Museum.

The first British Museum catalogues were contained in the suitably named *Naturalist's* (later *Zoologist's*) *Miscellany*, begun in 1790 by Shaw and illustrated by Nodder, then briefly continued by Leach. Gray's achievement was to produce the first adequate catalogues of major groups, starting with the reptiles. But how could a handful of underpaid, badly housed assistants contend with the flood of incoming material? Wallace's complaint to Darwin about the reaction to his claim to have brought 8,000 new species from the Amazon was met with commiseration for their overwhelming task and with a feeling that "too much systematic work and description blunt the faculties".

It was to produce more order out of chaos that the young Albert Gunther, initially a theology student at Tübingen, came to the Museum in 1857 to be led into "A suite of three half subterranean rooms, in which the spirit collection

was stored . . . The floor showed large damp patches. There was no ventilation. Non-inviting rheumaticky quarters at any time. But what did I care?" What indeed?—he regarded this first year working on the great *Catalogue of Fishes* (1859–1870) as the happiest in his life. His later friendship with Alfred Newton at Cambridge bore fruit for the enduring sustenance of all zoologists by the establishment in 1856 of the *Zoological Record*.

Carrying the weight of the Department during Gray's final years of illness, Gunther succeeded him in 1875. His relations with Owen are interesting. Initially very much the protégé, differences developed alongside increased responsibilities, notably in connection with the new Museum at South Kensington for which Owen was fully responsible although the final plans were made without the knowledge of the keepers (or indeed of Owen himself). This is no place to discuss either the architecture or the internal planning of a building into which Gunther had to move specimens for the custody of which he was responsible although "their selection and arrangement was to be left to the Superintendent."

That figure was almost immediately replaced by the first appointed director, William Flower, who also assumed the keepership when Gunther retired in 1895, to be succeeded in this capacity by Sydney Harmer with Tate Regan as a junior member of staff. Here this book ends—not far short of when zoologists of my generation came into the picture.   **C. M. Yonge**

Quantum theory

Quantum Theory of the Solid State. By Joseph Callaway. Part A: Pp. x+1–370+10. Part B: Pp. xiii+371–824. (Academic: New York and London, July 1974.) $29.50; £14.75.

THIS work is in two parts, the first containing an account of the formalism required to study solids and the second concerned with more specific problems. The book is at a level suitable for students with a good background in quantum mechanics and some knowledge of the experimental facts of the solid state.

Part A is divided into four chapters, the first on lattice dynamics being followed by magnetism at a phenomenological level. Chapters 3 and 4 can be viewed together as an introduction to the group theory, followed by the calculational methods needed to find the electron states (and from the symmetry standpoint the phonon states) in periodic crystals. Part B then treats impurities, the effect of external electric and magnetic fields on solids and transport phenomena. In the final chapter there is an introduction to many-body theory.

Professor Callaway, of course, has made important contributions over a wide area of solid state physics and the book reflects his deep understanding of his subject. I wonder, however, a little about his ordering of the material. For, although many-body theory is treated in depth only in the last chapter, on p. 22 we have the creation and annihilation operators, and on p. 41, also in the first chapter, the van Hove correlation function appears in its full quantum mechanical form. Nevertheless, even for the reader who does not struggle too hard with the more advanced detail in Chapter 1, there is a good deal of basic solid state physics concerned with phonons which can be learned from it. The references, which are at the end of each chapter, number about 60 on phonons. Three refer to work later than 1971 but the rest are from 1969 and earlier.

Chapter 2 begins with the Heisenberg Hamiltonian along with some discussion of how the exchange parameters can be calculated from first principles. Molecular field theory is then discussed at some length, followed by an account of antiferromagnetism. The spin wave problem is then tackled using the Heisenberg Hamiltonian, with the dependence of spin waves on an external magnetic field given some attention. The extensive and helpful (though pretty advanced) discussion of spin waves is followed by a discussion of neutron scattering from magnetic crystals. The Ising model is then given

a good deal of prominence, 15 pages or so being devoted to it. Phase transitions in ferromagnets are then treated, at about the same length. The discussion is scholarly, but in my opinion rather more advanced than is consistent with the author's remarks in the preface about the level of students it is written for.

In the chapter on symmetry, one of the nice features is the attention given to crystal field effects. The reader will probably find Chapter 3 on symmetry and Chapter 4 on band theory a good deal easier than the first two chapters. The chapter on energy bands has all the clarity one would expect from such an expert in the field; however, quite a bit of the discussion is available already in good sources. The discussion of the tight-binding method, which Professor Callaway has used to very good effect on the transition metals, is very helpful here. Finally, the central question of the way the crystal potential is to be constructed is discussed from the standpoint of density functional theory; Dirac-Slater exchange theory and gradient corrections to it are summarised.

Part B begins with the impurity problem, Wannier and also *t* matrix methods being dealt with in some detail. A nice account of the optical

theorem is to be found here. Effective mass theory, local moments, the coherent potential approximation and the Kondo effect are other topics treated. A chapter of about 100 pages then follows on crystals in external fields, including optical properties, and magnetic field phenomena, such as low field diamagnetism and the de Haas-van Alphen effect. Transport theory is discussed at length and the last chapter represents a useful introduction to many-body theory. Itinerant electron magnetism is treated in a very clear manner in this chapter, along with Landau Fermi liquid theory. Green's functions and diagrammatic analysis are also developed here.

The only general criticism I would want to level concerns the ordering of the material. The first two chapters might have been better placed after the consideration of symmetry and band theory. More discussion of many-body theory would have been helpful earlier in the book.

Without doubt, this is a valuable addition to the literature which I found very stimulating to read. The problems at the end of each chapter are helpful in assessing one's grasp of the material as one goes along, though some are pretty difficult.

N. H. March

Sound through the sea floor

Physics of Sound in Marine Sediments. Edited by Loyd Hampton. Pp. xii+567. (Plenum: London and New York, 1974.) $39.00.

THIS publication is a collection of 20 papers presented as the proceedings of one of four symposia organised by the Office of Naval Research to review current research activities and to discuss possible future trends in the subject of sound propagation in marine sediments. Edited by Loyd Hampton, the papers describe the research efforts of many of the world's leading authorities on a subject which is of increasing importance as the commercial exploitation of the natural resources that lie on, or beneath, the sea floor continues to expand into less accessible areas.

This collection of papers is presented quite informally and is therefore comprehensible to those not too familiar with all of the aspects of a relatively diverse field. The volume highlights the difficulties involved in obtaining accurate geophysical, acoustic and mechanical data from sediments in their natural environment. Such data have perhaps, not been given sufficient priority in the past, but the advent of large structures that are to be placed on the sea floor may change that situa-

tion. Current static loading tests may soon prove inadequate in solving settlement problems in the high energy environments that exist at sea. A possible alternative may be to consider the interrelationships between the required parameters and such physical properties as sound propagation, electrical resistivity and radioactivity. Acoustic properties will probably prove to be the most useful in this respect because of the fundamental relationships involving mechanical and physical properties of materials. With knowledge of these relationships, acoustic measurements could combine with the normal methods of site investigation which use standard acoustic techniques to provide more accurate interpretation as well as dynamic loading information. Continuous monitoring of the acoustic properties of sediments may also allow the measurement of the long term changes in sediment fabric and of the effects of severe weather conditions on sediment structure.

The presentation of the papers is of a general high standard, but as many authors are involved it is often necessary to compare data; this task would have been simplified if a standard set of units had been specified.

Peter G. Simpkin

Wrong word too early

Invertebrate Endocrinology and Hormonal Heterophylly. Edited by Walter J. Burdette. Pp. xviii+438. (Springer: Berlin and New York, 1974.) $21.50.

HETEROPHYLLY, as defined by the Oxford Dictionary, is a botanical term referring to plants having leaves of more than one shape. How then does this relate to invertebrate hormones? In fact, it does not. The editor has given a new meaning to the word, and uses it to describe the effects of hormones from one phylum on individuals of another phylum. From the title one expects to read about hormones from various invertebrate groups. But, in truth, the book considers only insect hormones, and of these, only ecdysone, juvenile hormone, and brain hormone.

The book contains contributions made to a meeting held at Houston early in 1971. Unfortunately, there has been a long delay between the submission of the manuscripts and the appearance of the book in late 1974, and, consequently, it is rare to find a reference later than 1970; in fast moving areas of research, such as juvenile hormone and ecdysone biosynthesis and degradation, many papers are already out of date.
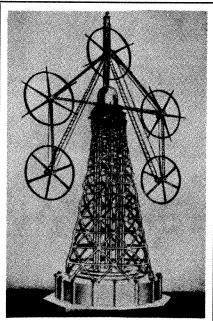
The book is divided into four parts. Part I, "Invertebrate endocrinology", deals with the control of metamorphosis, hormone assays, and hormone biosynthesis and degradation. The section starts promisingly with remarks by Wigglesworth on the use of hormones to probe basic problems in the regulation of growth and differentiation. The following papers include articles that cater to a general audience as well as those with very technical information aimed at the specialist. The series of papers on assay techniques, an example of the latter, are varied in their usefulness. The paper by Bjerke and Roller on juvenile hormone assays provides a broad, critical comparison of the common bioassay systems that were in use at the time. By contrast, the treatments of the brain hormone and ecdysone assays are much more limited in their scope and, accordingly, are of less value.

The second part considers primarily the isolation and distribution of various ecdysones. Since the original report in 1966 by Nakanishi and his coworkers of the presence of ecdysone-like chemicals in plants, the isolation and identification of these substances has, with a few notable exceptions, been carried out by workers in Japan. The articles in this section serve the valuable function of bringing together the data from the Japanese literature on the structure of the known phytoecyd-

sones (37 at the time the paper was written) as well as extensive listings of their plant sources.

Part III comprises six papers on the effects of vertebrate hormones on vertebrate tissues. Except for presumed parallels between the mode of action of these hormones on vertebrates, and that of insect hormones on insects, these papers bear little relationship to the rest of the book and seem to be quite out of place.

Hormonal heterophylly is the subject of the last part. From the various articles, it quickly becomes apparent that the effects of insect hormones on

Wind turbine designed in 1933. It was to have stood 300 metres high and would, according to its designer, have generated 50.000 kW. From *Power From the Wind*, by Palmer Cosslett Putnam. Pp. xii+224. (Van Nostrand Reinhold: New York and London, October 1974.)

vertebrate cells are neither striking nor profound. The effects of ecdysone on the growth of mammalian tumours and on human leucocyte chromosomes are at best slight and are certainly of questionable statistical significance. The insect hormones do cause metabolic changes in mouse liver, but this can scarcely be surprising in a tissue adapted for detoxification of foreign substances. Indeed, the fact that insect hormones have little effect on vertebrate tissues, as documented by the papers dealing with pharmacological screens of these hormones, is a point in their favour. Especially as one day they may be released into the environment as insecticides.

The editor of this volume states that it is the first concerted effort to collect and analyse responses of vertebrate, as well as invertebrate, tissues to insect hormones. In my opinion, it is a premature attempt.   **James W. Truman**

Old bones

Bones for the Archaeologist. By I. W. Cornwall. Revised edition. Pp. 259. (Dent: London, February 1975.) £4.50.

THIS is a revised edition of a volume first published in 1956. The section on conservation has some useful additions, the bibliography and the references have been improved and brought up to date and a very necessary index has been added. The bulk of the text and the illustrations remain the same.

When this book was first published the intention was to help the archaeologist make preliminary identifications of bones from archaeological sites at a time when much data had accumulated in museums awaiting the interest of some specialists from the natural sciences. It demonstrated successfully that most of this work could be done by the archaeologist himself if he was prepared to apply himself to learning the basic necessities of bone analysis. Many archaeologists, who were not entirely artefactually orientated, found it both necessary and relatively easy to add to their competence. There can be little doubt that some improvement in excavation techniques was one of the consequences of the publication of their book, as well as a reduction in the backlog of material awaiting analysis and identification.

The volume contains chapters on zoological classification and nomenclature, bone identification, field work, laboratory work and conservation, the estimation of age, sex and stature, and study and interpetation. For the tiro it even suggests logical methods of procedure. It is easily the best introductory work available at the present time and will guide many newcomers to archaeology to a useful and essentially practical expertise.

On the other hand, the weakest section is that concerned with study and interpretation. There is no clear indication of what purposes underlie the collection of bone specimens nor, in consequence, how bones should be collected on the site to give an adequate sample. It still remains that, in contrast to pollen analysis, data is atrociously collected during excavation by archaeologists with vastly different interests, techniques, knowledge and relevant expertise; there is still no discipline about archaeological collection. In consequence, samples obtained are commonly of minimal value and amenable to valid interpretation only at the coarsest levels. This fact could have been pointed out to advantage at a time when numerical analyses are increasingly fashionable. Excellent teaching as, for the most part, it is, Dr Cornwall's book stays with the vision of some 20 years ago.   **E. S. Higgs**

obituary

John E. Vance, a chemist who worked on processing of ores and the preparation of pure uranium compounds for the Manhattan Project, has died in New York at the age of 69.

Dr Vance was emeritus professor of chemistry at New York University and former head of the department. He joined the faculty in 1948 and retired in 1971. He had previously been associate professor at Yale, where he directed work on quantitative analysis, and crystal growth. In the 1940s, he was appointed by the State Department as senior staff member to Frederick Osborn, representative to the United Nations Atomic Energy Commission. Dr Vance's special concern was drawing up proposals for international control of atomic energy. At various times, he had served the army research office, particularly during the Manhattan Project which developed the atomic bomb in 1942.

Cyril Leslie Oakley, CBE, FRS, the distinguished bacteriologist, died on March 27 at the age of 77.

Professor Oakley graduated from University College, London, in Zoology in 1930 and held the position of experimental pathologist at the Wellcome Research Laboratories from 1934, becoming head of the Immunology and Experimental Pathology Department in 1947. From 1953, he was Brotherton Professor of Bacteriology at the University of Leeds, becoming Professor Emeritus in 1972. His own interests were in the fields of antibody production and bacterial toxins, and looked after those of others as President of the Association of Scientific Workers from 1956–59. Professor Oakley will, however, be primarily remembered as the editor of a number of scientific journals, including *Pathology and Bacteriology* (1956–68), *Medical Microbiology* (1968–71) and *Pathology* (1969–72).

Louis W. McKeehan, professor of physics at Yale from 1927–55, has died in Jamestown, Rhode Island at the age of 87.

After graduating from the University of Minnesota in 1908, Professor McKeehan taught there as an associate professor until the outbreak of war. At Yale, he directed the Sloane Laboratory of Physics and after becoming emeritus professor, he was research associate at the Laboratory of Marine Physics in New Haven and Jamestown. He had a lifelong association with the navy and had been assistant naval attaché in London during World War II and chairman of the scientific advisory board of Operation Crossroads, for the strategic bombing of Japan. His research included discharge in gases, ferromagnetism, submarine mines, slow-motion spheres in gases, scattering of cathode and β rays, and radioactivity.

announcements

Awards

George J. Todaro has been awarded the **Parke, Davis Award** by the American Society for Experimental Pathology for investigation of viral and genetic factors in the cause of cancer, in particular the viral oncogene hypothesis.

H. E. Ganther has been awarded the **Mead Johnson Award** by the American Institute of Nutrition for his work on the essential nutrient selenium.

Appointment

J. G. Williams has been appointed professor of polymer engineering at Imperial College, London.

International meetings

May 28–30. **Frequency control,** Atlantic City (Dr J. R. Vig, US Army Electronics Command, Fort Monmouth, New Jersey 07703).

June 2–5, **Information transfer in eukaryotic cells,** Montreal (Ms D. Mathieu-Thériault, c/o Dr R. Sinclair, Biology Department, McGill University, Box 6070, Montreal H3C 3G1, Canada).

June 7–18, **Solar terrestrial physics,** Colorado (American Geophysical Union, 1909 K Street, NW, Washington, DC 20006).

Miscellaneous

Cancer awards. The Cancer Research Institute Inc., is establishing Annual Awards in Cancer Immunology. The Institute will honour work judged to have been most critical in founding Cancer Immunology as we know it today, whether in reference to basic experimental research or to observations of more immediate clinical significance. Nominations to Mrs Helen C. Nauts, Executive Director, Cancer Research Institute Inc., 1225 Park Avenue, New York, New York 10028 (closing date July 15, 1975).

Cherwell–Simon Lecture. The lecture for 1974–75 will be delivered by E. W. Montroll, Director of the Institute for Fundamental Studies and Albert Einstein Professor of Physics in the University of Rochester, New York, on May 23, at 4.30 pm in the lecture theatre of the university museum. The subject will be 'Some quantitative aspects of social phenomena'.

Reports and publications
Great Britain

Parliamentary and Scientific Committee. Annual Report for 1974. Pp.16. (London: Parliamentary and Scientific Committee, 1975.)　　　　　　(112

Ministry of Agriculture, Fisheries and Food. Novel Protein Intelligence Unit. Bulletin No. 3: Leaf Protein —Current Developments in the United Kingdom. Pp.28. (London: Ministry of Agriculture, Fisheries and Food, 1975.)　　　　　　(112

Department of Industry. Report on Research and Development 1974. Pp.122. (London: HMSO, 1975.) £1.10 net.　　　　　　(122

The Association of Commonwealth Universities. Annual Report of the Council together with Accounts of the Association for the year 1 August 1973 to 31 July 1974. Pp.52. (London: The Association of Commonwealth Universities, 1975.)　　　　　　(122

National Institute for Medical Research. Report 1973/1974. Pp.iii+123. (London: Medical Research Council, 1974.)

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 277, No. 1274: Analysis of the Flexural Vibrations of Variable Density Spheroids Immersed in an Ideal Fluid, with Application to Ship Structural Dynamics. By R. E. Taylor. Pp.623–648. (London: The Royal Society, 1975.) £1.　　　　　　(132

Other countries

Anadémie Royale de Belgique. Mémoires de la Classe des Sciences. T.XLI, Fasc.6: Contribution à l'Etude de l'Atmosphère Terrestre Supérieure à Partir de l'Analyse Orbitale des Satellites. Par J. Vercheval. Pp.183. (Bruxelles: Académie Royale de Belgique, 1974.)　　　　　　(252

Cadmium and the Environment: Toxicity, Economy, Control. Pp.88. (Paris: Environment Directorate, Organization for Economic Co-operation and Development, 1975.)　　　　　　(262

La Theorie des Pulsations Internes de la Terre: Complements. Par Georges Dubourieu. Pp.47. The Theory of Internal Pulsations of the Earth: Further Evidence. By Georges Dubourdieu. Translated from the French by Louis Janssens. Pp.49. (Meudon: Collège de France, Laboratoire de Géologie, 1974.)　　　　　　(000

Alberta Research Council. Annual Report 1974. Pp.67. (Edmonton: Alberta Research Council, 1975.)　　　　　　(282

Taking stock of *Nature's* bread and butter

VERY occasionally we get our leg pulled about the words on our refereeing form. Our standards call for "topicality, brevity and plausibility". "You'll find this paper seductively plausible", purred an author in a letter of transmittal. "Plausible—yes; correct—no", responded a referee. So we went back in some diffidence to Fowler, who generally gets it right—

'**plausible** has moved a long way from its original meaning, "deserving of applause". Applied to a person it is always pejorative; a plausible man is one who obtains a credence he does not deserve. Applied to an argument the word has not travelled so far on the downward path: it may still be used of one that commends itself, though speculative.'

Continue to send us plausible papers. We will do our best not to send them to plausible referees.

● It is no doubt foolish to boast of the turn-round time for manuscripts, but since it forms the basis on which many scientists decide where to publish, here goes. Once a manuscript has been accepted, it will, on average be published within six weeks.

Statistics on the refereeing process are far more variable, of course, as it may take anything from a week upwards to satisfy ourselves and a referee that a paper should or should not be published. Not all papers go to referees. Some of those submitted seem to us more obviously suited to a specialised journal; and after taking appropriate opinions around the office —and often outside—we return such manuscripts as soon as possible. There is little point in seeking a referee's opinion and only then over-ruling it with our own opinion.

Of the remaining manuscripts, we seek a referee's views on all but a tiny fraction. The tiny fraction are not, as is widely believed, those written by very eminent men, but papers on matters where the facts are indisputable and delay would be positively detrimental to the community.

Some authors feel that sending a paper to a distinguished scientist for him to transmit does some good. It doesn't—it only profits the Post Office.

● One of the greatest problems encountered by sub-editors is that of ambiguity. This is exceptionally acute in a journal in which more than half the readers and many of the authors do not have English as their first language. English is undoubtedly an organic language capable of great flexibility and subtlety through the ease with which it handles nouns as adjectives, absorbs new words, possesses many words meaning almost the same thing and so on. Asset though this is, particularly in the spoken language, it does lead to difficulties for the reader—and not only the reader to whom English is a second language.

It is impossible to set down guidelines; ambiguities follow no rulebook. But here are some which may give some idea of the subtleties which readers are sometimes asked to unravel.

Jones is a solid state physicist (or soft rock geologist).

The equipment has provided extensive trouble free operation.

Smith's paper suggests a new concept (to Smith or to the author?)

Since Robinson made the observations, we have confidence in the theory.

Brown's work appeared to resolve the difficulty.

We shall hopefully repeat this work within a month.

The solution boiled momentarily.

(These last two demonstrate that the generation of ambiguous euphemisms for 'I hope' and 'in a moment' has effectively eliminated two words from the language.)

Some of these examples seem trifling to the English speaker; but the test we try to apply (not always with success) is whether the writing would be clear not only to someone not having English as a first language, but also to someone from a remote discipline reading out of curiosity.

● It is always tricky to know what references are really necessary; to the extent that we attempt at one and the same time to carry large numbers of short scientific reports and also to have them widely accessible we cannot avoid some conflict over the amount of referencing. But on the whole, we prefer relatively few references because anyone who finds interest in a paper outside his own field is presumably going to look and talk further. Particularly vulnerable to the sub-editor's pen are sentences that declare: "There has recently been great interest in this subject[1-11] and much work[12-25] is at present in progress".

● Many people secretly fancy themselves as advertising copywriters and would relish the opportunity to string together compelling but honest words to persuade others to buy cars, read books or (dare we say) to drink a certain brew or smoke a certain cigarette. And yet given an opportunity for self-advertisement, most scientists shy away from compelling but honest words with which to introduce their papers.

Nature has always avoided a summary at the beginning of letters partly for reasons of space, partly in an attempt to retain a certain directness in the reporting of early and often tentative results. But this should not inhibit anyone from writing a short and forthright first paragraph, capable of being understood by almost anyone. On occasions our sub-editors attempt to do this themselves for authors, but the response is often: "Well I didn't quite mean that". Which might, just once in a while, not stem entirely from our dim-wittedness but conceivably from a touch of opacity in the paper itself. □

Updating the Green Revolution
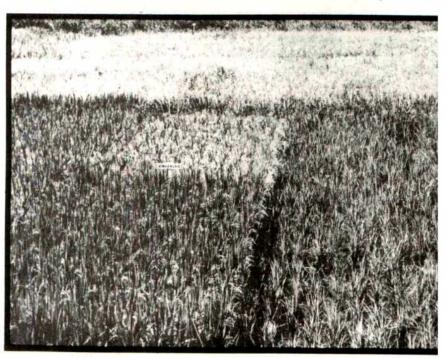
BY DAVID SPURGEON

PERHAPS the most important problem facing agricultural scientists in the decades ahead is how to keep production of staple foods like rice abreast of population increase in the Third World. In Asia alone, something like 5 million tons of rice will have to be produced every year to keep up with the demand. That means that new varieties and agronomic practices must spread, and that new lands must come into production. It also means that pests and flooding must be controlled or their effects minimised and that, in an era of inflation and rising prices, the costs of technological inputs such as fertilisers must be reduced.

One of the chief reasons that the success of the Green Revolution has been limited so far is that the total area in which the new seed varieties and agronomic practices were applied has been small. In the case of rice, the high yielding varieties were semi-dwarf types suitable only for shallow water regions (depth approximately 5–50 cm). These lands constitute only 25–30% of the world's total rice lands and even in these regions, of course, the new varieties are not grown exclusively. The Green Revolution has scarcely touched the other rice-growing areas—the uplands and regions of intermediate and deep water that together comprise 65–70% of the total available rice lands.

The International Rice Research Institute (IRRI) in the Philippines, where the new varieties and technology were developed, is at present working, in collaboration with rice scientists and growers elsewhere, to extend the Green Revolution to the majority of the world's rice-growing areas—and to solve some of the other problems that limit rice production. Some of them feel that rice production could be doubled throughout the world within the next 15 years through such methods. According to Dr Gurdev Kush, head of the IRRI's plant breeding department, the situation is not that gloomy when the available resources are considered. The problem is exploitation of the resources.

The really limiting factors in food production are political, Dr Kush believes, and in addition vast areas of Africa and Latin America have not been exploited. "I'm convinced," he says, "that if there's no major economic upheaval, and provided population control programmes are maintained, we can keep up with demand for the next 15–20 years."

One of the rice-growing sectors of the world being looked at with special interest by IRRI scientists contains the deep water regions, because these are among the most heavily populated anywhere. People have lived for centuries along the deltas of the mighty rivers of Asia—the Ganges, the Brahmaputra, the Godavari, the Irrawaddy, the Cheo Phraya and the Mekong—because of the adequate supply of water and the rich silt deposits.

Often, floating rice is the only food crop that farmers can grow in these areas, but the tall varieties found there are low yielding: for decades they have produced only 1 or 2 tons a hectare. Almost 10% of the rice land in Asia and Africa is planted with floating rices, whereas almost half the total rice land has tall non-floating varieties that are adapted to medium-deep water.

Last summer, the first International Seminar on Deep Water Rice was held at the Bangladesh Rice Research Institute north of Dacca—just after flood waters had destroyed much of that country's rice crop. The disaster served as an object lesson for the group, which surveyed both the flood damage and deep water rice culture from helicopters and boats, noting fields where semi-dwarf and even tall varieties were submerged and killed in fields adjoining flourishing deep water rice.

These floating varieties have developed genetic mechanisms that allow them to grow normally in deep water and to escape flood submergence—their stems elongate (for reasons not understood) as waters rise and their leaves float on the surface. Rice scientists are now incorporating this genetic characteristic into semi-dwarf and intermediate stature rices, hoping to develop high yielding varieties that can survive floods. Several thousand hectares of an IRRI strain known as IR442–2–58 are now being grown in West Bengal, India, where flood water often reach a depth of 1 m. More than 40 other crosses have been made between floating and semi-dwarf types and progeny are being tested in Bangladesh, India, Vietnam, Indonesia and Thailand. The headquarters for the deep water programme is at the Hunter Deep Water Rice Research Centre in Thailand.

"Not many scientists are really experts on deep water rice, despite the extent of the deep water areas and the huge populations they support," says Dr Mohammed Amirul Islam, Director of the BRRI. "This makes it all the more important that attention be focused on the urgent problems of deep water rice."

Other rice types being developed are varieties that will not elongate like floating rices, but will withstand submergence for a few days under flood conditions. These varieties would be useful where flooding is temporary: the floating varieties might lodge (topple over) as floodwaters recede. The need is illustrated by an incident in the Philippines in 1972, where about 230,000 hectares of rice land were flooded in early growth stages for four weeks, causing a crop loss of about $74 million.

Still other varieties of rice are being developed to adapt to the opposite of flood conditions: drought. About 10–15% of the world's rice-growing areas

Rice trials field in the Far East

suffer from insufficient water supply, for example in the uplands. Varieties tolerant to drought are being sought that will, nevertheless, recuperate quickly when the rains come, for areas of varied rainfall. Many regions of the world are too cold for the new varieties yet local rices grow there. Scientists at the IRRI are now gathering seeds from these rices and growing them in ice water to see which ones will survive and then to try to cross breed them.

The IRRI calls this programme of rice improvement the Genetic Evaluation and Utilisation Program (GEU). It brings together not only plant breeders but pathologists, entomologists, soil chemists and agronomists in order to identify and evaluate systematically rice genetically adapted to the most pressing constraints on rice production.

Besides the attributes mentioned, the programme seeks to incorporate these other factors into new high yielding varieties: resistance to insects, resistance to diseases, high protein levels and tolerance to toxic soils. In addition scientists are screening rice varieties to determine which use fertiliser and other agricultural chemicals most efficiently, and how best to deliver the chemicals.

It has been found, for example, that, if fertiliser is incorporated in a kind of mud pellet and placed near the plant's roots, about half the amount of fertiliser normally used will produce the same yield. In other words, the method doubles the efficiency of the chemicals. The difficulty is to package the fertiliser and insecticide together in a suitable way and to persuade industry to produce it.

Some success has already been ac-

hieved with disease and insect resistance. Early this year the IRRI named three early maturing rice varieties (IR 28, 29 and 30) as being resistant to a number of diseases and insects. They are the only known varieties highly resistant to grassy stunt virus disease, a serious problem in many countries. The varieties IR 28 and IR 29 are also resistant to tungro, bacterial blight and blast diseases; IR 30 is resistant to tungro and bacterial blight but moderately susceptible to blast. And all three varieties are resistant to brown plant hoppers and green leaf hoppers and moderately resistant to stem borers.

Furthermore, IR 28 and IR 30 mature in only 105 days and IR 29 in 115 days, compared with about 160 days for traditional tropical varieties and 130 for most high yielding varieties. These short seasons should enable farmers in some areas to adopt multiple cropping systems, thus cutting their risks. Varieties that mature in shorter periods are less likely to be damaged by insects or disease or weather simply because they are in the field for a shorter time. In addition, some farmers might fit in an extra crop this way.

Bad soil conditions prevent farmers from growing rice on millions of hectares. Felix Ponnamperuma, a Ceylonese soil chemist at the IRRI, visited Bombay and found that just 10 miles outside the city there were 100,000 hectares of land ideally suited to rice cultivation yet unusable because of salinity, whereas inside the city people were dying of malnutrition. This is flat, alluvial soil, and there is water throughout the year. There are about 10 million hectares of saline soils in India and Pakistan.

In Indonesia new lands are being opened up, says Dr Ponnamperuma, but they are largely peaty bogs. The country hopes to open up five million hectares in the next 10 years, but they

contain problem soils, and rice does not grow well on them. In all there are about 40 million hectares in the tropics composed of these marsh soils, mostly in South-east Asia.

Scientists now are studying the deficiencies of these soils—deficiencies that include almost all the major nutrients and some trace elements. Even when these elements are added they have found that rice growth is not as good as it should be, probably because of the presence of organic toxins. "So we want to breed varieties adapted to these conditions," says Dr Ponnamperuma. "We are trying to tailor the crop to the soil rather than the reverse."

Problems include acid sulphate soils (in perhaps 15 million hectares on which rice cannot be grown in Vietnam and Thailand); iron toxicity when certain soils are flooded (affecting 10–50 million hectares of rice soils), retarding growth and limiting yield; and zinc deficiency (affecting 500,000 hectares in the Philippines and large areas of Pakistan and India). The IRRI found in the Philippines that zinc deficiency sometimes prevented rice growth altogether, but that dipping seedling roots in zinc oxide and water was the answer. (Only $1 worth of zinc oxide per hectare (1 kilogram) often produced higher yields than 550 kilograms of complete fertiliser). And with zinc oxide seedling dip, any of the modern varieties of rice could produce twice the yield of the local varieties, even without any other fertiliser.

A third of mankind—1,300 million people—depend on rice for more than half their food. Ninety percent of the low income people of the most densely populated regions of the world rely on it, people whose average income is only $80. The efforts of the world's rice scientists will be crucial to their survival in the years ahead. □

Resistance ratings of IRRI varieties

Variety	Diseases				Insects			Soil problems			
	Blast	Bacterial blight	Grassy stunt	Tungro	Green leaf-hopper	Brown plant-hopper	Stem borer	Alkali injury	Salt injury	Zinc deficiency	Phosphorus deficiency
IR8	MR	S	S	S	R	S	MS	S	MR	S	MR
IR5	S	S	S	S	R	S	S	S	MR	R	MR
IR20	MR	R	S	R	R	S	MR	S	MR	R	R
IR22	S	R	S	S	S	S	S	S	S	S	MR
IR24	S	S	S	MR	R	S	S	MR	MR	S	MR
IR26	MR	R	MS	R	R	R	MR	MR	MR	S	R
IR28	R	R	R	R	R	R	MR	MR	MR	R	R
IR29	R	R	R	R	R	R	MR	S	MS	R	R
IR30	MS	R	R	R	R	R	MR	MR	MR	R	MR

R = resistant   MR = moderately resistant   MS = moderately susceptible   S = susceptible

Energy in the OECD

G. R. Bainbridge, Professor of Energy Studies and Director of The Energy Centre, University of Newcastle upon Tyne, reflects on the OECD report, Energy Prospects to 1985.

WHEN, in 1972, the council of the OECD initiated an assessment of energy developments and related policies it was anticipating that the projected growth of energy demand in the 24 countries constituting the OECD area would put increasing pressure on low cost energy supplies. It was particularly concerned about future supplies of oil from the Middle East, where 90% of the world's petroleum trade originates and where 55% of the proven natural oil reserves lie.

Moreover the USA, using a third of the 5,000 million tons of oil equivalent world annual energy consumption, was becoming increasingly an oil importer. Indeed only two OECD countries, Canada (trading oil and gas for coal) and Australia (trading coal for oil) have net total energy self sufficiency. So the pressure of oil demand would inevitably have important implications for the economies and policies of most OECD members, 15 of whom are dependent for 70% or more of their energy needs on imports, and only some of whom (the USA, Norway, the Netherlands and UK) could with sustained effort join the select group of those reasonably self sufficient in energy.

The concerted oil export restrictions applied by OPEC towards the end of 1973, followed by a dramatic increase in crude oil prices, came as a shock, emphasising the timeliness of the OECD energy exercise. It was clear that the prospects for a return to an era of relatively cheap energy had "dwindled considerably".

It is not surprising that the OECD assessors found themselves unable to provide probable, or target, projections of energy supply and demand, even for 10 years ahead, while initiatives were and still are being taken by the oil suppliers and consumers almost daily. They made, instead, a brave attempt to present in a qualitative way the principal likely determinants of future development of the energy sector. Increasing gross national production would be expected to require more energy, though perhaps not too much more if the trend towards improved energy-efficient processes, continues

and the likely substitution of capital and labour for more costly energy use occurs.

The price of energy had scarcely before been considered explicitly as a factor influencing energy demand, because prices were low and fairly stable. Now it had become the main one. In obtaining estimates of future energy use, oil prices rising from the early 1973 baseline of $3 (USA 1972 money) a barrel (7 barrels~1 tonne) to $6 and $9 were taken to provide a framework for investigating the implications for other economic factors and to identify possible problems that might confront the policymaker. Without criticism they could have gone even higher with the alternatives. During the period of the exercise, of course, dollar depreciation made those prices selected $3.60, $7.20 and $10.80 by the end of 1974. Inflation is only one of several factors listed in the recent Solemn Declaration of the Sovereigns and Heads of State of the OPEC member countries for consideration by them when adjusting crude oil prices in future.

By comparison with growing gross national product and primary fuel prices the other determinants of energy supply and demand tend to fade into insignificance, particularly in the next worrying decade of readjustment. Measures already taken or planned to improve environmental protection were found to tend to increase energy consumption, albeit by only a few percent. Although some of them have already been relaxed or left in abeyance to ease the energy crisis, efforts towards energy savings, if successful, could enable environmental protection measures to be resumed.

As might be expected the main part of the OECD report concentrates on the prospects for the provision of oil, coal, natural gas, electricity and nuclear power in the near future. The possibility of greater indigenous production within the OECD is considered particularly important, with nuclear fission, North Sea oil and gas, and rejuvenation of coal and its synthetic products being all seen to have useful parts to play.

In order to broaden the options for future choices, and to open up greater possibilities for the diversification of energy supplies, continuing research and development efforts directed towards exploitation of some of the renewable resources are recommended in the report. The contributions from solar energy and further geothermal energy were recognised to be expected in general beyond 1985. Research into the derivation of fuels from towns' wastes and the productions of methanol and hydrogen was also recommended, with thermonuclear fusion as the *pièce de résistance* for the long term. Little was

said of wind and wave power, or ocean thermal gradients, as an energy source, perhaps because the prevailing Southwesterlies and the Gulf Stream have, apparently been veering a little off course of late.

Anyone preparing to read this very informative OECD report should apply initial energy to acquiring a good magnifying glass, as the many and interesting tables in the key chapters 2 (Energy Demand and Supply Projections) and 3 (Economic and Environmental Implications) are printed minutely. Chapter 4, on Energy Conservation, is appropriately of below average length, but that glass is needed again for the vital table which analyses energy conservation possibilities; but the effectively blank four pages immediately preceding the chapter emphasise the scope there is for workers in the energy field to practise what they preach. More than 5% of the 224 pages in Volume 1 carry no significant point and the proportion is higher in Volume 2.

Because of the dominance of US energy consumption (and production) in any overall OECD energy trends, it was correct that the USA should be treated separately. In 1972 the USA was using 50% more primary energy than the 19 European OECD members together, and its indigenous production was three times greater than theirs. Separate consideration of Canada was also useful, for, although it uses only a tenth as much energy as the USA, its surpluses of oil and natural gas will increasingly be directed southwards. Japan (also separately treated) already imports more energy than the USA and in future will continue as a major competitor for the world's oil, coal and natural gas resources—unlike the USA, Japan has little scope for indigenous production and has a higher growth rate for both population and gross national product.

The EEC is included for statistical comparison in some of the tables of the report. The strength of the EEC compared with the OECD European 19 lies in the fact that the smaller grouping contains the major industrial countries, using well over 50% of the primary energy, producing over 70% of the European indigenous fuel supplies and with the better natural potential to improve on that position if pressed. The energy strength of the EEC might have been further improved by Norway, but that country chose through a referendum to opt out; it has indigenous North Sea oil and gas production potential of the same order as the UK, but a very much smaller population already well endowed with hydro-power resources.

The figure summarises historical behaviour and possible alternative trends

of future OECD energy requirements. Had world oil prices not increased, the doubling time for energy consumption would be running at about 15 years, some 4.9% a year. Although the OECD, with present energy consumption running at a little over 3,500 million tons of oil equivalent a year, accounts for about 70% of world usage, the developing countries outside the OECD group will tend to bias world growth to a higher rate and a shorter doubling time. With the oil import price increased by factors of 2 and 3, some energy savings are anticipated to bring OECD growth down to 4.3% a year and 3.8% a year respectively. Then, at 1985, the higher oil prices have depressed energy use (or encouraged desirable energy savings) to the extent of 7% and 12% respectively, on the total. The nuclear, coal and gas proportions are all increased for the higher oil prices, relative to oil.

Some of the immediate broad conclusions could have been deduced by intuition almost as easily as from detailed analysis, namely:

● The OECD share of oil is expected to decline to below 50% of total energy requirements from around 55%, though its use is still increasing steadily at 1985 and seems likely to have reached at least 2,800 million tons of oil equivalent a year, compared with 2,000 million tons of oil equivalent a year today. The increment alone is a factor of 4 higher than the anticipated oil yield from the North Sea.

● The natural gas share grows on balance, the percentage increment in Europe compensating for a relative decline in use in the USA. Although the percentage stays around the 22% mark, the annual use almost doubles from 600 to about 1,200 million tonnes of oil equivalent. It should be noted that natural gas is being increasingly supplied into the Eurogas grid from Russia and Holland; soon additional supplies will come north from Africa by undersea pipeline by way of Italy, supplementing present liquid natural gas trans-shipments.

● Nuclear power is expected to play a steadily increasing role, rising (including a small component of hydro) from around 4% of total energy now to some 8 to 10% by 1980 and 12 to 16% by 1985. As might be expected, therefore, the nuclear doubling time is of the order 5 to 7 years, below half that for total energy. This is significant in view of the long lead times for power station construction, and must depend on policy decisions already taken in many countries to press on more quickly with nuclear programmes in view of the higher oil prices.

●

● The use trend for coal is expected to increase again, reversing a steady decline after the Second World War. European coal resources are mainly in Germany (brown) and the UK (black) but the USA still has vast and relatively easily worked coal deposits. It is there and in Australia that the most likely prospect for greatly raising coal production arises, though in Europe an increase of 10 to 20% seems to be a sufficiently challenging and achievable target.
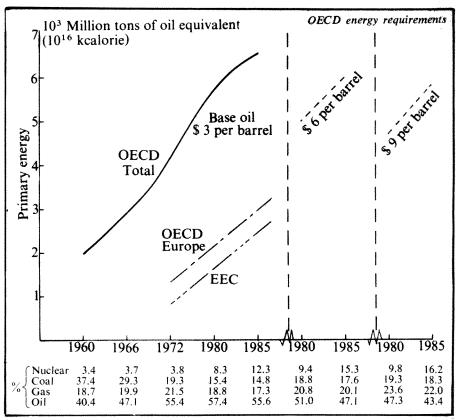
The analysis leading to the projections does not seem to have allowed for the probability that coal and natural gas prices are bound to rise to what the market will bear for these fuels, relative to the oil price, or that nuclear capital costs will escalate because of the effect of increased conventional fuel and labour costs on manufacture. These occurrences will depress the demand for fuels alternative to oil, and the supply of oil may exceed demand sufficiently for selling competition to re-enter the market. The report is right to suggest that, because the energy pattern of the OECD and indeed the world, is a dynamic one, continuing study to achieve a better understanding of it is worthwhile. For example, OECD oil imports in 1980 could easily be 70% higher than now if savings of energy use, substitution of other energy forms and restriction of national product outputs are not effected. Higher oil prices, if sustained, could, however, reduce the extra import demand to below 20% and cause even a reduction of the

amount of imported oil and expenditure on it, ignoring any oil suppliers' price adjustments to optimise their returns.

Actions recommended to OECD members to improve the arrangements for cooperation in energy and related fields, inter-regional (EEC and OECD Europe) and intra-regional (Europe–North America, Europe–Far East) if possible, seem laudable; emergency energy-sharing schemes, promotion of energy trade, energy savings schemes, energy research and development to ensure adequate supplies at reasonable cost all need work. Inflation, and the sorting out of balance of payments problems, are current international problems needing OECD support.

A bringing together of the oil-exporting and oil-importing countries in agreed policies for mutual and world benefit is another area recommended for OECD action, with joint studies of problems to find agreed solutions which will bring greater stability to the energy and money markets. The idea that scientific and technical assistance should flow from countries with the capability towards those that need to develop is one on which OECD and OPEC representatives should be coming close together.

In the UK Parliamentary debate on the EEC energy proposals early in February of this year it was concluded that as trade with our partners becomes more interdependent there is obvious sense in seeking to evolve coordinated energy policies. The OECD report firmly confirms that view. □

OECD energy requirements

10^3 Million tons of oil equivalent (10^{16} kcalorie)

Primary energy

Base oil $ 3 per barrel
OECD Total
$ 6 per barrel
$ 9 per barrel
OECD Europe
EEC

	1960	1966	1972	1980	1985	1980	1985	1980	1985
Nuclear	3.4	3.7	3.8	8.3	12.3	9.4	15.3	9.8	16.2
Coal	37.4	29.3	19.3	15.4	14.8	18.8	17.6	19.3	18.3
Gas	18.7	19.9	21.5	18.8	17.3	20.8	20.1	23.6	22.0
Oil	40.4	47.1	55.4	57.4	55.6	51.0	47.1	47.3	43.4

international news

EARLY last year, the revelation that Canada's National Research Council (NRC) had for many years served as a cover for the secret gathering of intelligence shocked many Canadians. It also led the then leader of the New Democratic Party, David Lewis, to propose shifting the NRC unit concerned (the so-called Communications Branch) into a more appropriate department, and Prime Minister Trudeau to promise that he would consider the suggestion.

A few weeks ago, the Canadian public learned that the question had indeed been considered: in fact the Communications Branch had been transferred to the Department of National Defence. The information came out in questioning of the Minister of State for Science and Technology, C. M. Drury, in the House of Commons.

But the matter didn't rest there. Questioning of Mr Drury (who, as it happens, is a former president of the Treasury Board) revealed that the budget of the branch could not be identified separately in the estimates, but instead was spread about in such a way as to make it impossible for MPs to find out how much had been spent

More about spying and the NRC

from David Spurgeon, Ottawa

on this sophisticated form of spying (the branch monitors electronics data signals and analyses them).

Mr Drury defended the procedure by saying it was necessary in order to make it difficult for Canada's enemies to learn about the operation. "The counter-intelligence movement . . .", he said, "must, in order to be effective, itself be under cover . . . To take a popular analogy, this is a bit like engaging in a poker game in which one man discloses his hand by putting it on the table and the other keeps his hidden and then proceeds to bet."

Walter Baker, Progressive Conservative MP, Ottawa, was disturbed because, as he said: "Members are to find out about security organisations by accident" (the matter came up in a CBC television programme last year) "and then be denied any opportunity to

ask questions about the scope and purpose of such activities or the amount of money to be spent on them."

It would be comforting to accept assurances that everything being done in the name of security is fit and proper, but recent history in other countries suggested otherwise, he said.

Mr Drury admitted that the need for secrecy presents democracies with a problem, but the problem was not peculiar to Canada. "The problem, from the administrative view, is how to seek [this] parliamentary approval [for security expenditures] without disclosing in a comprehensible and clear way what is being done, and how."

In the past, said Mr Drury, when members of the House felt they must know what was being done, they had been informed *in camera*.

To which Stanley Knowles, New Democratic Party MP from Winnipeg, replied: "The last *in camera* session of this House of Commons that I can remember took place in 1944." Mr Knowles said it is open to the government to keep things secret, but quite a different matter to distribute items through a number of estimates in order to distort the picture given to Parliament of how money is being spent.

The Speaker concluded the session by saying that the "question is extremely important and complex, and I would like to look at it carefully." In view of the fact that Mr Drury had also offered to tell the ministers what was involved *in camera*, "it would perhaps be best to ruminate over the Easter break and see what might develop." □

An interesting footnote was to be found in a series of recently published letters from Dr C. J. Mackenzie, acting president of the NRC from 1939–1943, to General A. G. L. McNaughton, the president, who was on leave of absence as commander of Canada's field forces. (*The Mackenzie-McNaughton Wartime Letters*, edit. by Thistle, M., with introduction and epilogue by Mackenzie, C. J., University of Toronto Press, 1975).

In one of these letters, dated December 6, 1941, Dr Mackenzie said: "There is one very interesting but very secret development which only one or two of us know about. We have organised under the research council a section on cryptography which has succeeded in breaking down codes and cyphers and doing a really good job. It seems an unusual activity for the research council but the intelligence officers of the three services, the Mounted Police and the officers of External Affairs asked me if we could organise such a unit, as they thought we could probably do it easier and keep

it under cover better than in any other way . . . We have an associate committee consisting of the intelligence officers of the three services, the RCMP, a member from External Affairs and myself. We have an expert cryptographer from England and I look after the business arrangements, finances, etc . . . We got into it in the first instance because our facilities were such that we could start a unit in a modest way to see whether or not it was a practicable thing to do.

"My feeling was that after we had done the organising and got the staff trained, an official unit could be set up by the government to operate under one of the services or External Affairs. After six months of trial, which a decision had to be made as to how it should continue, all of the members were most insistent that they would prefer it to be carried on under our auspices and that will be done, for a while at least. It is pretty far from research but it is a most important and useful service."

Letter from Japan

from Yoshinobu Kakiuchi, Tokyo

Two new inter-university research institutes, the High Energy Physics Institute and the Institute for Molecular Science, have recently been established in Japan. The first is located at Tsukuba some 35 miles from Tokyo. The place is known as an academic town, the intention being that it should centre on universities and various research institutes. The High Energy Physics Institute was the first institution to be brought into the area, followed by the Pollution Research Institute and the Institute for Inorganic Materials, both of which belong to the

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Science and Technology Agency. And a new national university (Tsukuba University), based on various new attempts to break with university tradition, has just started activities there.
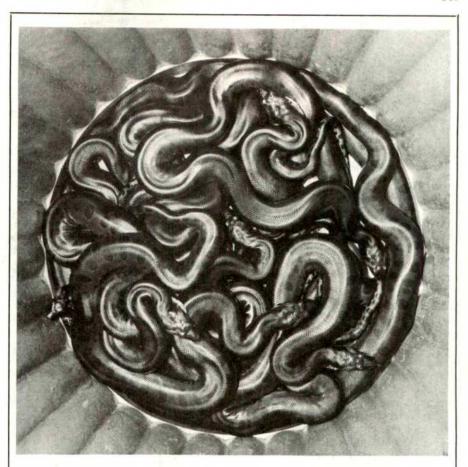
The Institute for Molecular Science is to be located at Okazaki 190 miles to the west of Tokyo, where it is also planned to build two other new institutes devoted to the life sciences.

The idea of the inter-university institute has been nurtured in Japan for some time. Soon after the Second World War, Japanese scientists began to think of establishing forums where they could exchange their ideas and cooperate in research of common interest. Nuclear scientists, for example, wanted to build an accelerator for nuclear research, but the cost seemed too large to be borne by a single university at that time. Therefore, the facility was supposed to be shared by researchers all over Japan. Traditionally, however, the national universities receive money from the government individually, which made it rather difficult to set up facilities to be shared on an entirely equal basis by several of them. The compromise was that institutes should be affiliated to a particular university, which is made responsible for its operation; but the facilities are available to the research scientists outside that university. At present there are more than a dozen of these so-called "institutes for joint use".

The establishment of these institutes raises a series of interesting issues, and naturally there are some difficulties. Universities in the main remain rather closed in structure, and the institutes for joint use are no exception. They usually open their doors only to scientists closely related to the research they cover, and this often causes friction. Of course, it is not impossible to promote inter-institutional joint projects, as is exemplified by the joint programme set up by the Institute for Nuclear Study and the Institute for Solid State Physics, both of which are affiliated to the University of Tokyo. There had been a good deal of spectroscopic work with electron synchrotron radiation in the past, but the spectroscopists involved wanted to build a storage ring to provide increased energy and radiation intensity. The ring has been successfully tested under the supervision of Professor Taizo Sasaki, of the University of Tokyo.

To escape more completely from the university strait-jacket, the idea of inter-university research institutes on a national basis, completely detached from the existing universities but still keeping a university's essential characteristics, were developed.

The Institute for High Energy Physics, for example, was established in 1971, at the recommendation of the

THE picture of young anacondas is taken from the Annual Report of the Zoological Society of London for 1974. In its annual report the Zoo records a rise of expenditure of 16% over last year, mainly on salaries and animal feeding stuffs. Unfortunately this coincided in 1974 with a fall in the number of visitors to both London and Whipsnade. But the numbers picked up again, apparently, after the arrival of the two giant pandas from Peking as a gift from the Chinese government; they arrived last September in a blaze of publicity.
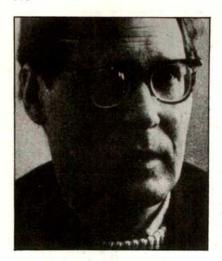
Science Council of Japan, the advisory body to the Prime Minister's Office on scientific affairs. The recommendation was originally made as early as 1962, on the basis of extensive studies of a possible proton synchrotron. The project was investigated in detail and was finally authorised by the Ministry of Education. This synchrotron produces energetic protons in two steps—the booster ring accelerates them to 500 MeV and the main ring (108 m in diameter) brings them up to $8 \sim 12$ GeV. The five-year plan presented to start with is to be modified to a seven-year plan, with an increase in the total budget from about £10 million to about £15 million.

According to Professor Shigeki Suwa, the director of the institute, there has been plenty of cooperation between scientists and engineers in the design and the construction of the accelerator. He also says that the institute is keen to open the way for technicians to achieve better positions in the institute through promotion. A storage ring of the colliding beam type, equipped with super-conducting electromagnets, is still on the drawing board, but is being designed to produce a 70-GeV proton beam. Preliminary testing of the super-conducting wires is progressing satisfactorily.

The building of The Institute for Molecular Science has just been started, and Professor Hiroo Inokuchi, of the University of Tokyo, is now responsible for supervising the preparation of its activities. The setting up of this institute was also recommended by the Science Council of Japan (in 1965) and it is scheduled to cover five major fields of research, ranging from molecular theory to cooperative molecular phenomena.

This institute will be administered along similar lines to the Institute for High Energy Physics, and a development worth mentioning is the establishment of a "technical development section", responsible for the design and development of new research instruments. The original five-year plan for the institute is covered by a total budget of some £6 million. ☐

Sergei Koralev: arrested in December

Moscow scientists under pressure

from Vera Rich, London

THE arrest and harassment of members of the Moscow group of Amnesty International highlights yet again both the involvement of a number of leading Soviet scientists with problems of human rights and the extreme disfavour with which these activities are viewed by the authorities.

Physicist Andrei Tverdokhlebov, the secretary of the group, was arrested in Moscow on April 18, on his way to work. The same day, the Ukrainian science-fiction writer Mykola Rudenko, a postal "associate member" of the group, was arrested in Kiev.

Another prominent member of the group, Dr Sergei Kovalev was arrested last December, as part of a KGB operation against the *samizdat* journal "The Chronicle of the Lithuanian Catholic Church". Dr Kovalev has since been charged with "anti-Soviet agitation and propaganda".

Other members of the Amnesty group, including the Chairman, cyberneticist Valentin Turchin, and Vladimir Albrekht, a mathematician, have had their apartments searched, and documents, including Amnesty material, confiscated.

The Moscow group of Amnesty International was recognised by the International Executive Committee of Amnesty last September. Following the usual procedure, the Secretariat assigned the new group three case-sheets, referring to prisoners of conscience in Sri Lanka, Yugoslavia and Spain respectively. The group as such, had no connection with the internal affairs of the Soviet Union, in accordance with the policy of Amnesty International which does not allow any of its groups to concern itself with prisoners in its own country.

Although certain of the members of the group, have, as private individuals, also been active as regards the defence of human rights within the Soviet Union (Tverdokhlebov and Turchin have, on occasion, been co-signatories to the "open letters" for Dr Andrei Sakharov), it seems clear that it is not only their actions as private individuals, but also the existence of the Amnesty group which has earned official disfavour. The latest reports from Kiev indicate that although Rudenko has been released, his continued freedom is conditional; he has had to sign a promise not to leave Kiev, has been warned that he is awaiting further investigation and trial, and that his Amnesty activities "compound his crime" against the Soviet Union.

A statement from Academician Andrei Sakharov, addressed to the General Secretary of Amnesty International and to the "international public", described the arrests and harassments as "an affront to international public opinion and an attack against legality and democratic and humane principles to which this organisation and its members in the USSR invariably adhere."

Soviet space achievements celebrated

In spite of the recent setbacks to the manned Soyuz programme, Soviet Cosmonautics Day, April 12, was celebrated with the usual progress report on Soviet achievements. Although, on the practical side, the festival was marked only by the launch of that most routine of Soviet spacecraft, a Kosmos (number 726), this deficiency was more than offset by the official and quasi-official speeches.

At a celebration meeting held in the Central Theatre of the Soviet Army in Moscow, Academician Boris M. Petrov spoke of the year's work in tones of unqualified achievement. On such an auspicious day, nothing was said of the problems with the Soyuz–Salyut projects. The stress was entirely on the potentialities of the Salyut programme "for long term experiments in space with human participation".

Special emphasis, too, was placed on the expansion of Soviet participation in international space projects. "Successful cooperation" is at present going forward between the Soviet Union and the USA, France and India, and this cooperation on "major projects" will be extended during 1975 to include Sweden. The Interkosmos (joint COMECON) programme also progresses steadily, the latest satellite in the series being Interkosmos 13.

Within the purely Soviet framework, more than 50 Orbita communications satellites have now been launched, and a new one is planned to bring television to the workers on the Baikal–Amur Railway (one of the present major Soviet prestige engineering projects.

Although the official speeches at such a celebration must, of necessity, be laudatory, Academician Petrov's plaudits are, in fact, substantiated by a year of solid routine achievement. Although the Soyuz setbacks have captured the headlines, valuable preparatory work has gone forward on the Soyuz–Apollo plans (the major difficulty still, apparently, being one of linguistics). The Franco–Soviet "Araks" experiment, operating from magnetically antipodal bases in the Arctic and the Kerguelen Islands, is proceeding steadily with its programme of investigations of the terrestrial magnetic field. Although unfavourable meteorological conditions in the Antarctic have made it impossible, to date, to carry out the main part of the experiment—the injection of electrons into the magnetosphere to create an artificial aurora—some valuable, if routine, observations of the natural aurora have been carried out. Interkosmos 13, with its payload of Czech and Soviet instruments, was likewise devoted especially to magnetospheric and polar ionospheric research, a programme which is also being supported by a team on the drifting ice-flow polar station North Pole 22. A few days after the celebrations, on April 19, the first Indian satellite (named Ariabata after a fifth-century mathematician) was successfully placed in orbit from a Soviet carrier, achieving, *inter alia*, the distinction of being the heaviest first satellite of any country (360 kg).

Cosmonautics Day is, however, not only the time for an annual stocktaking of Soviet space achievements; it is also an occasion for press speculation and "informed" interviews on future plans.

Much of this material is necessary speculative and non-committal. "To prove, for example that there is not and was not life on Mars would be no less important than to find life there", is a typical comment of this type. Nevertheless, from these "supporting" interviews it would seem that long term planners are at least toying with the possibility of recovering soil specimens from the nearer planets. The combination of a Lunokhod-type vehicle with telecontrolled manipulators and a return craft of the Luna-16 type is considered "very promising" for this purpose. And, with an eye to the more distant future, the Institute of Medico-Biological Problems of the Ministry of Health is already working out ecological recycling and life-support systems for eventual long term space flights. ☐

DRINKING water throughout the United States is contaminated with trace amounts of organic chemicals, some of which are suspected carcinogens, according to a survey of drinking-water quality in 79 cities. The survey, carried out by the Environmental Protection Agency (EPA), indicates in particular that chloroform is an almost universal contaminant—it was found in samples of drinking water from all 79 cities, in amounts ranging from 'barely detectable' to 311 parts per billion.

Moreover, a more intensive survey of drinking water in five cities—Miami, Seattle, Ottumwa, Philadelphia and Cincinnati—has turned up evidence of a wide range of chemicals in the water supply. Thirty-five different organic chemicals have been detected in Miami's drinking water, for example, and thirty-six have been found in Philadelphia's.

Just how great a hazard to health those contaminants may be is a matter of some debate, but Russell Train, the Administrator of the EPA, said last week that "even at these low levels, the chemicals are a matter of some concern". The National Academy of Sciences is now evaluating the health hazards, and a special advisory committee has been established by the EPA to help determine national standards for drinking-water supplies.

The EPA survey looked specifically for six different chemicals in drinking water — chloroform, bromodichloromethane, dibromochloromethane, 1, 2,-dichloroethane, bromoform and carbon tetrachloride. Chloroform was the most ubiquitous, but bromodichloromethane and dibromochloromethane were also present in almost all the samples tested.

Officials of the EPA have expressed surprise at the widespread occurrence of the contaminants, and Train summed up the findings thus: "Our basic conclusion . . . is that the problem of organic chemicals in public water supply systems exists throughout the country."

The survey does not identify the sources of the contaminants but there is strong evidence that the chlorination process, which is the most widely used purification method for drinking water, may itself be responsible. A survey of raw, untreated water showed no trace of the six chemicals in 30 of the cities and extremely low concentrations in the other 49. But at least one of the chemicals was present in every sample of treated water that was tested.

The EPA's findings are likely to rekindle the smouldering debate about whether or not there is a safe level of exposure to carcinogens below which no health hazards are encountered. It should be noted, however, that the food and drug laws in the United States are based on the assumption that there is no such no-effect level, since they specify that no chemical which raises cancer in test animals can be added to foodstuffs. The extension of that concept to drinking water would, however, prove difficult, for Train noted last week that although several promising processes for water treatment are being developed, "we simply do not have a single proven method for dealing with all aspects of the organics problem".

The immediate effect of the EPA's

Washington seen

by Colin Norman

findings is likely to be a boom in the market for bottled water, but tests by both the EPA and the Food and Drug Administration have shown in the past that many varieties of bottled water are no 'purer' than tap water.

● The job market for scientists and engineers who have PhD degrees is likely to deteriorate during the next 10 years, according to a survey soon to be released by the National Science Foundation (NSF). According to projections of the likely output from graduate departments of universities in the United States, there will be between 375,000 and 400,000 scientists and engineers with PhDs by 1985, but only about 295,000 are likely to be employed in jobs related to science and engineering.

Those predictions "indicate a trend toward increasing imbalances between supply and utilisation . . . of science and engineering doctorates, possibly in some outright unemployment", the report states. But it adds that although the magnitude of the unemployment is difficult to project, it "is expected to be relatively small since individuals with doctorate education are likely to find some sort of employment—possibly in non-science and engineering activities or in underutilisation of their training".

The NSF's projections indicate that the widest divergence between supply and demand for PhDs is likely to be found in the social sciences, whereas the life sciences are likely to be almost in balance.

A prime reason for the deteriorating job market is the fact that there is expected to be a decrease in enrolment in universities and four-year colleges, which are traditionally the biggest employers of science PhDs. Consequently, the projections indicate that a smaller proportion of PhDs will be engaged in academic research and development in 1985, and that as many as 20% will not be involved with science and engineering at all.

In fact, the projections suggest that almost half of all new job openings for PhD scientists and engineers in the next decade will be in activities unrelated to research and development. The report suggests that such a shift "has major educational implications for institutions as well as for students".

● President Ford has at last signed an executive order to set the seal on the ratification by the United States of the Geneva Protocol, which outlaws the first use in war of chemical weapons. The executive order, which establishes a "national policy" under which the United States has renounced the first use of herbicides and riot control agents in war, had been bottled up for almost three months in the Justice Department, and formal ratification of the protocol by the United States was being held up until Ford signed it. The order, in short, allows the Administration to hang on to its belief that herbicides and riot control agents are not strictly covered by the protocol, but the United States has renounced their first use anyway.

● The Energy Research and Development Administration (ERDA) has backed off from a plan to store radioactive waste material from nuclear power plants in temporary aboveground storage facilities, and has decided that the whole matter requires more study. The move, announced last week by ERDA Administrator Dr Robert Seamans, follows widespread criticism of the plan by a number of environmental organisations. Although the temporary storage scheme has not been dropped entirely, ERDA may well move straight to a permanent disposal operation, consisting of burying the wastes deep in a salt mine. A promising site is under study in New Mexico, but it will take many years to carry out tests there. The original idea was to store the waste material in temporary facilities until a permanent disposal site had been found and tested, and ERDA had asked Congress for $5 million to start constructing the above-ground facilities next year. Seamans said, in a letter to the Joint Committee on Atomic Energy, that no decision would be made until 1976 about how the waste disposal operation should proceed.

correspondence

Soviet Jews

SIR,—The wave of repressions against Jews seeking permission to emigrate to Israel has recently increased considerably in the USSR.

We scientists, who are being forcibly kept in the USSR against our wills, wish to express our opinion about the present deteriorating situation. We realise that the Soviet authorities now respond to the sympathy and public opinion of the West, and especially of the USA, in terms of what they can gain politically and economically. The Soviet government has found that the West reacts actively to the voices of the persecuted who are deprived of elementary human rights; they therefore try to deprive these people of their voice and to turn them into obedient instruments of Soviet policy.

So the main attack is directed against people whose protests are considered by the authorities to be the most undesirable. Thus Nashpits and Tsitlenok are now charged with participating in a 12-second-long demonstration of protest against the refusal of the Soviet government to issue visas to them. As recently as three years ago such action would have, at the worst, led to 15 days detention for "disturbance of public order".

The other participants in this demonstration were warned that if they set a foot wrong again their fate will be the same as that of Nashpits and Tsitlenok (now sentenced to five years' exile).

The magazine *Jews in the USSR*, created by Professor Voronel and dedicated to the scientific analysis of different aspects of the cultural life of Jews, was claimed at the trial of the writer Vladimir Maramzin to be against the law and anti-Soviet.

Nowadays, also, participants in the scientific seminar founded by Professor Voronel three years ago are persecuted severely.

● The present leader of the seminar, Mark Azbel, was recently told by the KGB that the important thing is not that he is a professor of physics, but that he is a lieutenant in the military reserves and subject to call-up for military service. It is ironic that a man aged 43, who has never been called up, should now, after 2½ years of waiting for a visa and 3 months in hospital, be needed in the military camps.

● Recently Dr Viktor Brailovskii was called to the KGB where he was threatened with the charge of "insulting a policeman" during a demonstration on February 24. But he was not involved in the demonstration.

● Dr Aleksandr Lunts was warned by the KGB that he can be charged as a traitor to the Motherland.

● In Kharkov unknown persons tried to break into the flat of Professor Leonid Gerber.

● Dr Mikhail Shepelev received seven summonses to military offices and a summons to appear before the police for so-called "parasitism" (being unemployed).

● Eltan Finkelshtein has been called to the military offices again and again.

● After being fired from his job, Dr Vainer was ordered to begin work in 15 days. But as it is impossible for a Jewish *refusnik* to obtain work in his profession, he will have to take a menial job.

● Dr Mikhail Mikylinskii and Dr Eugenir Yakir are charged with parasitism as well, in spite of the fact that they were dismissed from their previous jobs.

So, although nobody has been directly charged for participating in the seminar, it now seems too dangerous to be involved in it. And it is the only means of scientific contact for 8 professors, 24 doctors and 18 bachelors of science from eight cities.

The scientists are persecuted for scientific activities as they were in the Middle Ages. This is the true picture of KGB activities to stop the voices of Aliya (the movement for the repatriation of Jews to Israel).

It is hard to predict what will happen if the authorities achieve their aim. If protests cease, the government will be able to say that people no longer want to emigrate.

Whether Russian Jews will play a further part in changing the relationship between the USSR and the USA, and whether they will continue to be repressed is an open question; in any case, if their voices are silenced, the Soviet government would be quite free in its suppression of emigration.

We have too few opportunities for preventing such a sequence of events. We do not know how to convince the Soviet authorities that the humane attitude towards emigration is the only possible way in our time.

It is now abundantly clear that the mass attack against Soviet scientists who participate in Professor Mark Azbel's seminar has been instigated by the KGB in preparation for the 250th anniversary of the Academy of Sciences of the USSR. (The anniversary session was unexpectedly postponed last year because of elections to the Supreme Soviet.)

We are so anxious for scientific contact that we will be happy to speak at and to listen to as many lectures as possible. But such things are impossible, according to KGB logic. So the spirit of the participants in the seminar must be broken before the Anniversary.

We ask all people who are interested in helping the persecuted to help us—to raise their voices in defence of emigration, in defence of our natural human rights.

MARK AZBEL
VENYAMIN FAIN
ILYA PYATETSKII-SHAPIRO
VIKTOR BRAILOVSKY

. . . it is interesting to note that reports from Sweden and Norway state that during the night of March 29–30 last, a heavy rain of ashes or sand took place from the west coast of Norway to the Swedish frontier; the whole of the country was covered with grey dust to such an extent that from a pint of snow more than a tablespoonful of residue was left after the snow had melted. Some chemists of Christiania have examined the ashes, and one of them, Prof. Waage, states that the dust consists of little, irregular, but sharp-edged grains, almost all colourless—some few are of brown colour—and they consist principally of silicates. Acids extract some lime, iron, and alumina from their powder. The professor thinks it likely that the dust originates from an eruption in Iceland. This view is confirmed by a mineralogical investigation made on another sample of the dust at the Christiania University, by Profs. Kjerulf and Fearnley; they recognised the dust to consist of fragments of pumice-stone which is identical with the Hecla pumice-stone. According to Swedish newspapers, some traces of the dust-fall were observed even in the vicinity of Stockholm. Prof. Kjerulf also thinks it highly probable that an eruptiin took place in Iceland. The distance from the Iceland volcanoes to the Swedish frontier is about the same as that from Mount Etna to the Baltic.

from *Nature*, **11**, 515, April 29, 1875.

news and views

The nucleosome: subunit of mammalian chromatin

from Benjamin Lewin

EACH eukaryotic chromosome contains a very long duplex molecule of DNA, five classes of histone protein whose total weight is about equal to that of the DNA, a lesser weight of a somewhat larger number of nonhistone proteins, and a small amount of RNA. Little evidence has been available to show how these components may be organised at the molecular level, although it has for some time been apparent that DNA and protein form a fibre of regular dimensions which is condensed into the very compact structure of the mitotic chromosome and presumably is relatively less tightly folded into interphase chromatin. Two types of chromosome fibre were identified by DuPraw and Bahr[1] in electron microscopy of chromatin prepared by the critical point drying method (drying on a grid from CO_2); the basic thread folded into the human chromosome seemed to be a fibre of 250–300 Å, which in turn was generated by the folding of a fibre of diameter 50–100 Å. Although critical point drying may alter the dimensions of chromatin organisation, this suggested the general concept of a single continuous fibre folded upon itself many times, with more than one level of organisation.

The existence of a regular repeating unit in both interphase chromatin and metaphase chromosomes of Chinese hamster cells was suggested by Pardon et al.[2,3], whose X-ray diffraction studies suggested the presence of a supercoiled fibre of diameter 100 Å with a pitch of 110–120 Å. By introducing a new method for visualising chromatin structure, Olins and Olins[4] were able to suggest that the chromosome fibre might have the type of structure typical of beads on a string. Following the procedure first developed by Miller and Beatty[5], interphase nuclei of rat thymus

or liver or of chicken erythrocytes were diluted into water; the nuclei swell and break open, the contents are fixed in formalin and then centrifuged on to a grid. Spherical particles, called ν bodies, of about 60–80 Å in diameter can then be seen, connected by a thin (15 Å) filament. Individual ν bodies could be visualised in less stretched region of the fibre; a fibre of greater diameter was generated when the ν bodies were more closely packed together.

Histones

Another line of approach to resolving the structure of chromatin has been developed from analysis of histone properties. Histones are separated on acrylamide gels into five distinct classes, which fall into three groups according to the relative contents of the basic amino acids lysine and arginine in which these proteins are rich. Two different nomenclatures have been in use to describe these five classes; since both serve as a reminder of the erratic progress made in separating histones and now seem somewhat less than satisfactory in their relationships, I shall use a third nomenclature which was proposed recently[6] (see table).

The lysine-rich histone class H1 displays microheterogeneity and consists of several closely related proteins. This class is the most easily removed from chromatin and its removal allows the preparation to be solubilised; several studies of the physical state of chromatin depleted of lysine-rich histones have suggested the tentative conclusion that H1, as the least tightly bound, may be concerned with surface properties, perhaps with holding together a chromosome fibre consisting of the other components[7].

The two classes of slightly lysine-rich histones, H2A and H2B, show some conservation between species according to the criterion of gel mobility. The two classes of arginine-rich histones, H3 and H4, both show extensive conservation, with virtually identical amino acid sequences between organisms as unrelated as cow and carp (H3) and cow and pea seedling (H4); patterns of modification are similar but differ in detail between species. (The strict con-

servation of sequence makes these the most conserved proteins known.) Such striking suppression of variation suggests that the entire sequence of each of these histones is involved in its function and that this function may be virtually identical in all eukaryotes. A function common to such different species presumably must be structural, so that this implies that there may be common features in the molecular organisation of all eukaryotic chromosomes[7].

The tendency of histones to aggregate during extraction has been a complicating factor in analysing their function for some time. Kornberg and Thomas[8] suggested that this aggregation might be due to the denaturing conditions that were employed and therefore extracted histones by a milder method (in 2 M NaCl–50 mM Na acetate). When this histone preparation was fractionated on Sephadex, three groups of proteins could be identified: one peak contained a complex of H4 and H3; another contained a complex of H2A and H2B; H1 did not complex with any other histone. A tetramer containing two molecules of each arginine-rich histone ($H3_2$–$H4_2$) was obtained by applying ammonium sulphate precipitation and using a cross-linking agent; little of this tetramer was found when histones were extracted by the more usual acid and solvent procedure. The slightly lysine-rich histones also form an oligomeric complex (H2A–H2B), although its nature could not be so well defined. When the $H3_2$–$H4_2$ tetramer was mixed with the H2A–H2B oligomer, no reaction occurred as judged by the use of a cross-linking agent.

When the tetramer and the oligomer were mixed with DNA, however, Kornberg and Thomas found that they formed the same X-ray diffraction pattern as that displayed by chromatin. Based upon these observations, Kornberg[9] suggested a model for chromatin in which the basic structure is formed by the interaction of DNA with the $H3_2$–$H4_2$ tetramer and H2A–H2B oligomer; the lysine-rich histones H1 and the nonhistone proteins do not participate. The equal weights of histone and DNA present in chromatin

Histone nomenclature

	Original schemes		New scheme[6]
Lysine-rich	f1	Ib	H1
	(f2c	V	H5)
Slightly lysine-rich	f2a2	IIb1	H2A
	f2b	IIb2	H2B
Arginine-rich	f3	III	H3
	f2a1	IV	H4

correspond to about one histone each of the slightly lysine-rich and arginine-rich classes per 100 base pairs, with the lysine-rich H1 being present in only about half the molar amount. Following this equality, Kornberg proposed that the H3₂–H4₂ arginine-rich histone tetramer is associated with two copies each of the slightly lysine-rich histones H2A and H2B and with 200 base pairs of DNA; this forms the basic repeating unit. The lesser amount of H1 would correspond to one protein molecule for each repeating unit. By postulating that the arginine-rich tetramer forms the core of the repeating unit, it is possible to explain why these two histones are the last to be dissociated from DNA by mild extraction procedures and why they are the most conserved; the role of the slightly lysine-rich histones H2A and H2B might be to determine the spacing of the tetramer along the length of the fibre. This would generate a flexible structure, able to fold in the manner that must be necessary for compression within the chromosome.

Nuclease digestion

Support for this model is provided by several experiments which show that nucleases may cleave chromatin at regularly spaced sites, presumably the sites between adjacent repeating units. An early observation was that of Hewish and Burgoyne[10], who found that an endogenous nuclease in rat liver digests the DNA of chromatin when the nuclei are incubated *in vitro* in the presence of calcium and magnesium ions. When the DNA was purified, it formed a series of bands on acrylamide gels, suggesting that sites at regular intervals are available for cleavage. Clark and Felsenfeld[11] similarly found that staphylococcal nuclease is able to digest about half the DNA of chromatin to form fragments with an average length of 100–110 base pairs; in a more detailed report, Axel *et al.*[12] digested duck reticulocyte or calf thymus chromatin with this enzyme and found DNA fragments to be produced predominantly in the size classes 45–60 base pairs, 100–130 base pairs, >200 base pairs. Duck reticulocyte chromatin reconstituted *in vitro* from its components gave a somewhat similar result, except that the >200 base pair peak was more pronounced; the source of the DNA proved to be irrelevant, for the duck chromatin proteins were able to confer the same support against degradation upon any DNA.

Particles also have been obtained by digestion of DNA. Sahasrabuddhe and Van Holde[13] found that brief digestion with micrococcal nuclease converted chromatin into very compact particles sedimenting at 12S. Oosterhof, Hozier and Rill[14] more recently have prepared 11S particles by fragmenting calf thymus chromatin with DNase II; these particles contained DNA of a length of about 400 Å (125 base pairs). Less extended periods of digestion generated DNA in multiples of this length, of 250, 375, or 500 base pairs. Noll[15] found that more than 85% of the DNA of rat liver chromatin can be converted to subunits sedimenting at 11S upon digestion either with micrococcal nuclease or with the endogenous enzyme; milder digestion allows dimers of 16S to be seen. The 11S particle contained DNA of a length of about 205 base pairs; fragments of 405 and 605 base pairs, obtained upon the milder digestion, presumably correspond to dimers and trimers. The buoyant density of the particles of 1.45 g cm⁻³ in CsCl corresponds to a protein : DNA ratio of 0.77; all five histones and some non-histone proteins were present. Some DNA of 170 based pairs in length also was produced, probably as a product of degradation of the 205 base pair fragment, suggesting that shorter pieces of DNA can result from breakage of the basic unit.

In spite of some discrepancies between the size of the particle and the length of DNA contained in it, these experiments all support the general conclusion that there may be a sub-unit of chromatin which forms a distinct particle and contains a fixed length of DNA.

Electron microscopy

Results supporting the general model for histone–DNA association proposed by Kornberg and confirming that the length of DNA in the repeating unit is about 200 base pairs have been reported by Oudet, Gross-Bellard and Chambon[16]. In a direct visualisation of chromatin structure, chromatin of chicken liver was treated with 700 mM NaCl to dissociate the lysine-rich histone class and so give a preparation that is more readily dispersed; centrifugation through a discontinuous glycerol gradient in 600 mM NaCl then gave two fractions of chromatin, one more dense than the other. Both fractions gave the appearance of beads on a string when examined by electron microscopy, with particles of average diameter 128 Å connected by a fibre of 15–25 Å. Oudet *et al.* termed these particles 'nucleosomes', in analogy with the ν bodies; the connecting thread appears to be DNA since it is of an appropriate diameter and disappears after digestion with micrococcal nuclease. The less dense peak contained tightly packed nucleosomes separated by long stretches of free DNA; the denser peak contained only tightly packed nucleosomes. To exclude the possibility that nucleosomes might have been formed by rearrangement of histones in salt solution, selective degradation with trypsin also was used to prepare chromatin depleted of lysine-rich histones; this gave tightly packed nucleosomes of 124 Å connected by a thread of 18–30 Å diameter. To confirm that this structure represents that of chromatin *in vivo*, another technique was used; chicken liver nuclei were lysed directly on to the surface of a water drop on top of a grid. This revealed a very compact structure consisting of tightly packed 130 Å diameter nucleosomes.

By degrading the free DNA of the chromatin preparations that had been depleted of lysine-rich histone, it was possible to obtain strings containing a few nucleosomes, at later times converted to single nucleosomes. The nucleosome seems to sediment at 10.5–12S and represents a spherical particle with an average protein : DNA ratio of 0.97. Gel electrophoresis showed the presence of equal amounts of H2A, H2B, H3 and H4 histones. When the protein of the nucleosomes was digested with proteinase K, the isolated DNA could be visualised by electron microscopy and had an average length of 667 Å (197 base pairs). These results are consistent with the X-ray diffraction studies that suggested a diameter of 100 Å and repeating length of 110–120 Å; differences in hydration may explain the discrepancy in the spacing. The larger diameter of nucleosomes compared with ν bodies probably derives from differences in fixation procedures, for Oudet *et al.* found that 1% formaldehyde reduced the diameter of the particles to 96 Å.

The nucleosomes disappeared, of course, when the four remaining histones were dissociated in 2 M NaCl from chick erythrocyte chromatin that previously had been depleted of lysine-rich histones. When the chromatin was reconstituted by progressive decrease of the salt to 400 mM NaCl, particles of diameter 131 Å formed, connected by a thread of 15–25 Å. The only difference apparent in this preparation compared with those of chromatin was that somewhat greater lengths of free DNA connected the nucleosomes. Adenovirus DNA or phage lambda DNA gave the same structure when reconstituted with the four histones of calf thymus, a strong indication that the sequence of DNA is irrelevant to the formation of this structure. All four histones are essential if nucleosomes are to be reconstituted.

An indication that a regular repeating unit may also be constructed in another way is provided by the recent observation of Lohr and Van Holde[17] that treatment of nuclei of *Saccharomyces cerevisciae* with micrococcal nuclease generates bands of DNA of definite multiple sizes, 135, 275, 400, 520, 650 base pairs. The

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behaviour of yeast chromosomes at mitosis differs cytologically from that of chromosomes of higher eukaryotes and their content of histones is smaller : only three histone bands are found on acrylamide gels, H1 seems to be absent and it seems likely that H3 may also be missing. In this case the regularly repeating unit must have components different from those of mammalian chromatin.

The basic unit of mammalian chromatin thus seems to be a complex of diameter about 125 Å; this contains about 200 base pairs of DNA that are compressed about five-fold in length, and possesses two molecules each of histones H2A, H2B, H3 and H4. The DNA presumably is recognised as a polynucleotide duplex chain. The role of each histone within the nucleosome remains to be established; however, it seems likely that application of techniques analogous to those used to investigate the self-assembling ribonucleoprotein structure of the ribosome may prove profitable. That nucleosomes of all higher eukaryotic chromatins may be constructed very similarly is implied by the lack of nucleotide sequence specificity in the DNA component and by Kornberg's proposal that it is the highly conserved arginine-rich histones, H3 and H4, which interact the most intimately with DNA. Presumably the nucleosomes cannot be identical, however, since there may be differences in the slightly lysine-rich histones, H2A and H2B, and it would be interesting to determine the relationship between the mammalian nucleosome and the structure of the repeating unit of yeast, which may lack H3. The relationship between nucleosomes in

mammalian chromatin presumably is established at the molecular level by protein interactions involving the lysine-rich histones and nonhistone proteins; a system in which to investigate the role of lysine-rich histones may be the avian erythrocyte, in which H1 is replaced by a new lysine-rich histone, H5.

One question that is highlighted by these results is the role of modifications (methylation, acetylation, phosphorylation) that histones seem to suffer in the cell, sometimes on a cyclic basis. Modification is species specific; the extent of modification of H3 and H4, for example, varies in those species in which these proteins have been sequenced. There have been speculations that histone modifications might be imposed to allow histones to bind to DNA sequences with sufficient specificity, but the *in vitro* reconstitution and simple repetitive nature of the nucleosome argue against this conclusion. Perhaps modification becomes attractive as an evolutionary mechanism for permitting changes in the histones or parts of histones whose amino acid sequences are strictly conserved; its role remains to be established.

The lack of nucleotide sequence specificity in the architecture of the nucleosome implies that it is with any higher orders of organisation that an explanation must be sought for chromosome ultrastructure. The compression of DNA within the chromosome or interphase chromatin is many times greater, of course, than within the nucleosome subunit. It is therefore important to establish in what manner nucleosomes may be packed together. If lysine-rich (H1) histones are engaged

in some such general role, than it is the nonhistone proteins which must be responsible for variations in ultrastructure, including the differences between euchromatin and heterochromatin that may affect comparatively large chromosome regions, the existence of specific bands in mitotic chromosomes, and the activation of specific genes (presumably transcription could not be accomplished within the nucleosome which would therefore have to be disrupted). Recognition of specific DNA sequences clearly must be accomplished by regulator proteins and the structure of the nucleosome poses an old question in new guise: how are DNA sequences recognised within the nucleoprotein structure?

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What are tumour-specific antigens?

from D. Allen L. Davies

IN the spring of 1900 Paul Ehrlich, Director of the Prussian Institute in Frankfurt am Main, addressed the Royal Society with these words: "The idea has already been mooted by v. Dungern, of attacking epithelial new formations, particularly carcinoma, by means of specific 'anti-epithelial sera' ". Incidentally he also mentioned Metchnikoff's hope of attacking the symptoms of old age with anti-phagocyte serum; the latter has not been followed up with any success as far as I know (or was the paper turned down by *Nature*?). The former has been followed up but without the degree of success needed for clinical usefulness. In spite of this there are still generally great hopes for the future success of cancer immunotherapy and for reasons which are easy to grasp.

Today's cancer treatment is almost

wholly by one or more of three approaches: surgery, irradiation and chemotherapy. Though progress and improvements are continually being made in these areas, especially in relation to particular kinds of tumours, nobody believes that in any of them are substantial improvements likely, of such a scale that cancer would no longer constitute a personal threat of disastrous consequences. If tumour-specific antigens are a characteristic feature of all kinds of neoplastic cells, then they provide a marker to serve as the specific target for an immune attack which could be increased, sustained or artificially engendered for therapeutic use. In this case progress of more general applicability can be imagined.

Paul Ehrlich continued thus: "But even if in the immediate future no

great practical success is attained, we must remember that we are only at the beginning of a rational investigation of properties of cells which hitherto have been far too lightly regarded". If over the last 75 years that rational investigation can be said to have been undertaken, what do we have to show for it? We have vast accumulations of data, the conviction that cancer immunotherapy is still a favoured hope for the future, and the impression that the subject is much more complicated than originally envisaged. We could sympathise with Ehrlich if he were now to think that progress has been slow, but some rules have emerged.

There are, perhaps, three taxa: (1) tumour-associated antigens (carcinoembryonic antigen, α-foetoprotein, and so on), (2) those coded in viral genes (which are not necessarily exposed on

the tumour cell surface) and (3) new surface antigens resulting from intervention of abnormal DNA. This last group, but also some of group (2) are attractive targets for immunological attack, but it is not yet decided if all neoplastic cell surfaces have such new antigens. Authors adhering to one school of thought freely refer to 'non-antigenic' tumours; the other school attribute these to instances where the test methods were of inadequate sensitivity. There are certainly stronger and weaker tumour antigens as there are strong and weak histocompatibility (H) antigens. Tumours obey the transplantation rules (except under bizarre experimental conditions) implying that their H antigens are not grossly changed. From the practical point of view the allogenic situation is most revealing when enhancing alloantiserum blocks the recognition event, abrogating the prompt rejection of a histoincompatible tumour and allowing free growth.

Much early literature bears on H antigen changes on tumour cells (especially in mice) but these studies have not led to any clear guidelines. On page 713 of this issue of *Nature*, Invernizzi and Parmiani compare a non-antigenic spontaneous mouse tumour and an immunogenic carcinogen-induced tumour of the same (H–2^d) genotype to show that the latter expresses an H antigen belonging to other (H–2^b and H–2^k) mouse strains, and they believe this to be the true nature of the tumour-specific antigen. The suggestion is made that the tumour antigen possibly resulted from "random mutations at the level of histocompatibility genes". It is especially important to know if such a relationship exists between H and tumour antigens because there is something special about H antigens as targets for immune attack. Not only are they of prime importance in allogeneic situations, they are also the principal target for cellular immune attack even between different species. This surprising information is provided by Lindahl and Bach (*Nature*, **254**, 607; 1975). The two contributions make good reading for those working on immunotherapeutic approaches, especially as there is a resurgence of interest in tumour-directed antibodies as carriers of drugs (Davies and O'Neill, Eleventh International Cancer Congress, 1974). This is on the basis that the cellular response of the host has failed, that it cannot be replenished from an allogeneic (histoincompatible) source and antibody is not powerful enough, alone, to wreak sufficient damage on the target.

We should beware of generalising to simple rules such as that tumour antigens are derepressed or mutated H anti-gens. Such a suggestion was made, for example by Martin, Esber, Cotton and Rice (*Br. J. Cancer*, **28**, Suppl. 1, 48–61; 1973), but for at least one particular tumour, tumour-specific and H antigens are physically separable molecules (Davies, Baugh, Buckham and Manstone, *Eur. J. Cancer*, **10**, 781–786; 1974) and Yeferof and Klein (*Expl Cell. Res.*, **88**, 217–244; 1974) show that H antigens and tumour-specific antigens do not co-cap. Aberrant H antigens derived by mutation or derepression should co-cap using suitable H-directed antibody. On the other hand there are cases where, as tumour antigen increases, H antigen decreases (Haywood and McKhann, *J. exp. Med.*, **133**, 1171–1187; 1971); and there are other variations on this general theme.

It is a sobering thought that whereas it would be convenient, and even promising from a practical point of view, if H antigens and tumour antigens had the relationships claimed, we do not actually know the natural physiological role of H antigens and their polymorphism. Among about ten possibilities listed at a recent meeting the favoured role was part of a cell surface recognition structure. One might conclude that a career in cancer research is still a safe one, not likely to be interrupted by an actual cure for the disease.

Hedges as relics of ancient woodland

from Peter D. Moore

MANY conservationists, particularly those with a botanical bent, are agreed that hedgerows in Britain act as an important reservoir for wildlife, though some feel that their importance may have been overrated especially as far as birds are concerned (see *Nature*, **249**, 514; 1974). Hooper, a leading botanical champion of the hedges, has stated that as many as 50 species of plant could be threatened with local extinction as a consequence of the current agricultural practice of hedgerow removal (in *Flora of a Changing Britain*, edit. by F. Perring, 58; Clarsey, 1970).

Hooper has also pioneered research into the relationship between plant species richness and the age of hedges, finding that the two are generally positively correlated. This fact can, however, be interpreted in two ways; long-established hedges may have had time for invasion by more species, or, alternatively, old hedges may often be relic strips of rich, ancient woodlands. If the former explanation is correct then it would imply that hedges could act as chan-nels along which plants migrate, and, if this is so, hedges can be regarded as doubly important, since they provide the means by which reinvasion of isolated fragments of woodland can occur.

Pollard (*J. Ecol.*, **61**, 343; 1973) coupled a study of hedge floristics in Huntingdonshire and the Soke of Peterborough with an historical analysis of their origins. His results agreed with the findings of Hooper as far as age and richness were concerned, but he also showed that the richest hedges were those which could be traced back through documentary evidence to old woodland edges, often associated with parish boundaries. Some shrubs were entirely confined to woodland relic hedges, such as field maple, *Acer campestre*, dogwood, *Thelycrania sanguinea* and hazel, *Corylus avellana*. This latter finding is rather surprising when one considers the rapidity with which hazel colonised the early post-glacial land surface of north-west Europe (see Deacon, *New Phytol.*, **73**, 1055; 1974). The concept of hedgerows as corridors for plant colonisation began to look unlikely.
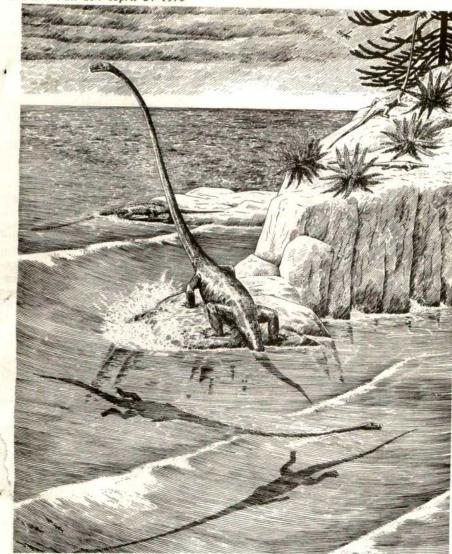
Helliwell (*Biol. Cons.*, **7**, 61; 1975) has dealt this theory a further blow in his work on hedgerows in Shropshire. Association analysis of his site data split first into base-rich and acidic subsets, and subsequently separated off hedges rich in woodland plant species. These are considered to be relic woodland fragments. Of course the use of a classificatory system of analysis renders it inevitable that a strict division of sites should result. But it is surprising that some invasion of modern hedges has not occurred if they do provide migration routes for woodland plants. This would have produced a continuum of richness.

These conclusions in no way deny the value of hedgerows as objects for conservation; it is the emphasis in determining site priority which should be modified in their light. Hedges of considerable antiquity, due to their richness, remain worthy of conservation effort. The case for maintaining recently planted hedges as aids to botanical recolonisation, however, becomes rather tenuous.

The longest-necked lizard?

from Barry Cox

THE Middle Triassic reptile *Tanystropheus* is one of the most peculiar fossils ever found. The adults are up to 6 metres long but with the head and the enormously elongated neck together contributing nearly one half of the

Adult (foreground) and young (top right) *Tanystropheus*, according to Wild. From *Schweiz. paläont. Abh.*, **95**, 1–162; 1974.

length of the animal. Even more surprisingly, there are only 12 neck vertebrae, and most of the elongation is confined to numbers 3–11, some of which are nearly twice the length of the skull.

Tanystropheus has been found in Switzerland, Italy and Germany. A long and fully illustrated account of it, based on 25 more or less complete skeletons, has now been published by Wild (*Schweiz. paläont. Abh.*, **95**, 1–162; 1974). The characters of the skull, and the apparent presence of autotomy joints in the tail, suggest that despite its extreme specialisation, *Tanystropheus* was a very early branch of the lepidosaur (lizard and snake) stock.

Interpreting the anatomy of this reptile in terms of its mode of life obviously presents many intriguing problems. *Tanystropheus* is found in deposits of the Tethys Sea, and a number of adaptations suggest that it was a marine predator. The hind foot is enlarged and fan-shaped; it was probably webbed, and provided the main propulsive force. The forelimbs and neck could have been used to steer the

spindle-shaped body. Wild suggests that the long neck was flexible, like those of plesiosaurs. This seems a reasonable assumption, despite the small number of neck vertebrae, and some of the articulated specimens certainly show a considerable amount of curvature of the neck. The cervical ribs are thin and extremely long, some extending alongside more than four vertebrae, and Wild suggests that they acted as an elastic brace to strengthen the neck. He states that preserved stomach contents show that adult *Tanystropheus* fed on cephalopods and fish, biting and grasping with their simple, conical teeth. Wild points out that the shallow seas around tropical Triassic Europe, with many reefs and lagoons, probably contained a rich fauna.

The young *Tanystropheus* lived a very different life, according to Wild. He states that small specimens of two of the three species are unknown, and that small specimens of the third species (*T. longobardicus*) have never been found in purely marine sediments. Wild points out that specimens of this last species that are less than about two

metres long have three-cusped teeth. Such teeth are found today in some insectivorous lizards and in the marine iguana *Amblyrhynchus*. He then suggests that the young *Tanystropheus* were terrestrial, and that they stretched their long necks upwards to catch insects on the wing! But, though even the smallest specimens have an elongated neck, this would seem to confer little advantage on an insectivore. In any case, the vertebrae of the trunk show no sign of the large neural spines that would be needed for attachment of the large muscles necessary to hoist the heavy neck into the air—and that are, for example, found in sauropod dinosaurs. Perhaps *Amblyrhynchus*, which is a marine herbivore, provides a better clue to the use of the tri-cusped teeth of *Tanystropheus*, whose young may have fed on seaweed in near-shore waters. If so, this peculiar animal, like the crocodiles today, changed its diet during its life, but not its habitat.

Mad hatter's tea party among X-ray sources

from G. T. Bath

THE world of galactic X-ray sources produces a continuing supply of Lewis Carroll characters. Transient, short-lived X-ray sources, which flare up and then disappear (leaving behind not even a grin) are now a well established class. These flaring sources seem to resemble the optical flaring outbursts of novae, but in the X-ray region of the electromagnetic spectrum. The recent discovery of a further flaring source by the Ariel V satellite groups at Mullard Space Science Laboratory and at the University of Birmingham (Ives, Sanford, and Bell Burnell, *Nature*, **254**, 578; 1975; and Eyles, Skinner, Willmore, and Rosenberg, *Nature*, **254**, 577; 1975) has however added a new problem to exercise the theorists' ingenuity.

Until now the periods associated with X-ray sources seemed to be of three types. First, very short-period regular pulses of a few seconds. These are thought to be due to axial rotation of a magnetic neutron star, which is itself a member of a binary system. The radiation in the pulses is then produced by accretion heating of infalling matter at the magnetic poles. The accreted matter is lost by the stellar companion, which is being slowly cannibalised by the gravitational attraction of the binary neutron star component.

This binary structure itself then provides a second period—the period of the binary orbit—which is generally of the order of a few days in the

sources with which we are at present familiar. A third period, which only occurs in the extraordinarily complex system Her X-1, is the 35-day period on which the X-ray radiation in this source seems to be switched on and then switched off, like a regular recurring X-ray flare.

But the new transient source, which was only visible for about ten days, seems according to the Mullard group to have a 6.75-minute period present in the signal. The problem for the theorists will now be to fit such a period into a binary, mass transfer model. If it is the rotation period of the accreting star it would be extremely, and indeed uniquely, long. If it is the binary orbital period then it would be extremely, and excitingly, short—demanding a very small size for the companion as the source of accreted material. But perhaps, like the 35 day on–off period of Her X-1, it will require a completely different and

individual explanation. If other X-ray sources can be taken as an example it will not be long before a plethora of theories are produced trying to account for this totally unexpected new observation by the Mullard group.

Two recently published optical observations of the X-ray source Cen X-3 by Petro and by Osmer, Hiltner and Whelan (*Astrophys. J.*, **195**, 705 and 709; 1975) add a note of caution and sobriety to the world of X-ray sources. The first of these is a series of photoelectric observations of the variations in brightness of the Cen X-3 binary system about its orbit. The second is a study of the spectral type of the optical, mass-losing star. Both conclude that those values for the masses of X-ray binary stars which in the past have been determined on the basis of fitting the spectral type (that is, the appearance of the star's spectrum) and the luminosity (the star's intrinsic

brightness) to theoretical stellar models can be seriously in error. This is extremely important in the present search for black holes in X-ray sources. For it has been claimed that the masses of the compact X-ray sources in several systems must be greater than the theoretical maximum possible mass for a neutron star. But these two papers demonstrate conclusively that one must be extremely careful in determining the orbital parameters of such X-ray binary systems. Simple-minded arguments based on our general understanding of single-star structure may be seriously in error when applied to the pathological X-ray binaries.

Nonetheless, this work on the Cen X-3 system does not dispose of the problem presented by the system Cyg X-1, in which the X-ray source is almost certainly more massive than the critical neutron star mass. Like the grin of the Cheshire cat this conclusion does not seem to want to fade away. The evidence for the existence of a black hole in the Cyg X-1 system is still, as yet, neither substantial enough to confound the sceptics, nor weak enough for them to dismiss it. Demonstration using the techniques of these two papers that Cyg X-1 was not a unique system would have helped in resolving this critically important issue. Now we must wait either for the sceptics themselves to fade away, or for further dramatic news from present, and future, X-ray satellites.

More from Ariel V

by John Gribbin

FURTHER early results from Ariel V were reported at a press conference on April 17, organised by the Science Research Council to coincide with the publication of the papers describing the source dubbed 'Cen Xmas' (*Nature*, **254**, 577 and 578; 1975). The UK satellite has in fact already produced two important results apart from the Cen Xmas source. The first is a general comparison with the Uhuru surveys: so far, with 30% of the first Ariel V survey completed at a sensitivity 3 to 5 times better than the Uhuru 1971-2 surveys, it seems that 16 of the Uhuru sources have disappeared in the past few years. Nineteen new sources have already been found, one (the brightest for a time) being a transient with a lifetime of only a few weeks. This evidence for a changing X-ray sky highlights the need for Ariel V (and its successors) to continue sky survey programmes.

The second major news item came from Peter Willmore (now at University of Birmingham) who announced that an investigation of the galactic centre region showed a bright X-ray source in February, and that this source had definitely not been detectable as recently as November 1974. This might not seem too remarkable in view of the frequency with which other sources seem to be popping in and out of view, but of course the galactic centre is a particularly interesting region where events that we can only observe through a dust veil,

darkly, seem to be happening at many wavelengths.

The most up to the minute news came from Ken Pounds of the University of Leicester, reporting a second Leicester experiment which "within the past few days" has "shown positive evidence for spectral lines near 6.6 keV and 2.1 keV" in the supernova remnants Cas A and Tycho's Nova. Claims in a release distributed at the conference that "if confirmed, these will be the first positively identified X-ray lines from a cosmic source" may seem a little exaggerated to those who remember the days when Sco X-1 was the only known non-solar cosmic X-ray source; it depends, perhaps, on the definition of "positively identified", but there has long been quite convincing evidence that iron lines have been detected on occasion in the flux from Sco X-1.

But that does not detract from the importance of these observations, which suggest an abundance of heavy elements in the remnants several times greater than the cosmic norm, nicely in agreement with the generally accepted view that all elements except hydrogen and helium are synthesised in stars and distributed through the galaxies by nova and supernova events.

Where next for Ariel V? Peter Sanford (Mullard Space Science Laboratory) says that the satellite should have a useful life of a couple of years, so the story is far from over.

Meteors by television

from David W. Hughes

NEW techniques are always welcome and meteor scientists have acquired one: television. The time-honoured method of observing meteors—standing outside on a clear moonless night and gazing intently at the sky—still provides many useful results. About ten meteors per hour can be seen; the eye can detect meteors down to about the fifth magnitude (produced by particles with masses in excess of 0.1 g) in the direct line of vision. The field of view is a cone with an apex angle of around 120° but sensitivity to the meteor is strongly dependent on its position in the field of view: the possibility of a meteor far from the direct line of vision being seen decreases drastically with decreasing magnitude.

This field of view restriction can be alleviated by using a camera such as the Baker Super-Schmidt meteor camera. This has a low *f* number (0.8), a circular field of view 55° in diameter, uses high speed film and can photograph

down to fourth magnitude meteors. If used in conjunction with rotating shutters the meteor velocity can be calculated. Persistent glowing trains left by bright meteors have been studied using an objective shutter and taking successive exposures at two-second intervals, slewing the camera by 0.3° after each exposure to separate the images.

What advantages does television have over these older techniques? First, it is considerably more sensitive; Clifton reported in the *Journal of Geophysical Research* (**78**, 6511; 1973) that magnitude nine meteors can be detected using his SEC (secondary electron conduction) vidicon system coupled to a single stage image intensifier. Up to 180 meteors per hour were seen in a field of view 13° by 16°. An image orthicon system which could detect stars of the same limiting magnitude (11th) only recorded half this rate because it was less able to detect fast moving objects.

The second advantage is the high degree of time resolution. The television cameras effectually take a photograph of the meteor every 1/25th of a second. The video recording thus contains a nearly complete record of the train's movement, its growth and subsequent decay and the light intensity as a function of distance along the train. A comparison of the 1/25th-second time interval with the two-second interval of the slewed Super-Schmidt underlines the importance of the new technique. If two spaced cameras can be used a light intensity against height profile can easily be obtained for all meteors detected by both. A large number of these profiles, for meteors of differing velocities and from different meteor showers, should help sort out meteor ablation, fragmentation and structure problems.

More results of television observations have been reported by Hawkes and Jones (*Mon. Not. R. Astr. Soc.*, **170**, 363; 1975). They compared the inferred mass distribution and incident flux of the observed meteors with values obtained previously using radar techniques. Radar can detect trains produced by meteoroids of similar mass (that is $>10^{-4}$g) but measures the electrons produced by meteoroid ablation and not the de-exciting atoms and molecules. Radar suffers from an awkward selection bias inasmuch as the initial radius of the train and its speedy radial diffusion can lead to destructive interference in the reflected beam. As both initial radius and diffusion are height dependent the meteors which ablate high up could go undetected. There is also the underdense–overdense radio reflection transition around third magnitude. Here the equations governing reflected power change. Changes in flux and mass distribution index around

third magnitude could be caused by transition problems and not be inherent in the incident flux. Fortunately the television system does not suffer from any of these drawbacks. Increases in height and range simply decrease the observed light according to the inverse power law. The luminous meteor train being optically thin does not undergo the third magnitude transition, from surface reflection to reflection by all the train electrons, suffered by radar.

Hawkes and Jones conclude that television meteors with magnitude between three and six have a mass distribution index of 2.02, very close to the values obtained by radar in this range. Also the incident flux is, within experimental uncertainty, the same as that observed by radar. It therefore seems that the initial radius bias is of only minor importance in radar back-scatter work, the height of maximum ablation not depending as strongly on mass as previously supposed. The diurnal variation of influx is in qualitative agreement with theory with however a subsidiary maximum around 01 h local time. This supports the original observations of Clifton. Statistical analysis of meteor train lengths indicates that the very faint meteors observed by the television systems (fifth to seventh magnitude) have vertical train lengths of about 6.7 ± 0.7 km, in good agreement with Verniani's work (*J. geophys. Res.*, **78**, 8429; 1973) on faint radio meteors—once more indicating that these small meteoroids (mass $\sim 10^{-4}$g) are fluffy dustballs and not solid and compact pieces of rock.

The television system does have problems. Clifton, assuming all meteors had a spectral class of A0, found that his system detected all meteors brighter than magnitude 6.4, but only 50% of the meteors brighter than 8.1. This problem is also encountered by Hawkes and Jones who find the sensitivity of the television system decreasing as they move out from the centre of the field of view. Even more serious is the velocity dependence of the observations. The fainter the visual meteor the slower it has to go to be detected. The sytem is therefore biased towards bright fast meteors (the peak train intensity is proportional to the product of the mass and the fourth power of the velocity). But television detection of meteors is here to stay and will be put to many exciting uses. Only about 40 hours of observation are needed to observe enough meteors to sort out the long outstanding problem of the third magnitude transition. And it is possible to observe the whole magnitude range from about 7 up to -1 using television alone, instead of the hotch-potch of underdense and overdense radar, telescopic, photographic and visual techniques used previously.

Status report on the inner planets

by John Gribbin

ON April 11 the Royal Astronomical Society, the Royal Meteorological Society and the Geological Society held a joint meeting for the first time. Though the meeting was entitled 'Mariner 10 Results on Venus and Mercury', it included results on other planets and data obtained from other sources.

DISCUSSING the structure and composition of the clouds of Venus, G. Hunt (Meteorological Office) described the evidence for the presence of sulphuric acid in the cloud tops. In spite of Mariner 10, however, it is still far from clear what lies closer to the surface of the planet; Hunt plumped for a two-layer structure with an upper aerosol layer separated from a deeper cloud layer by a cloud-free region, but confessed that the field is "still wide open" for alternative models.

Some of the greatest interest in the Mariner 10 Venus pictures has centred on the dramatic evidence supporting the view that the Venus atmosphere rotates with a four-day period, producing V-shaped cloud formations. John Hinch (University of Cambridge) was persuaded to bring out of semi-obscurity a two-year-old model of the movement of the Venus atmosphere which, from rather simple assumptions, produces 'predictions' which seem, in the light of Mariner 10, almost too good to be true.

This is basically a 'moving flame' model, in which the heat of the Sun passing over the atmosphere (the moving flame) leads to a thermal tide with resulting circulation. Hinch's lucid discussion was memorable for the smooth way in which some rather unpleasant mathematical modelling was interpreted in physical terms which sounded quite plausible. By assuming a uniform temperature within the atmosphere, large magnitude fluctuations, a thin viscous boundary layer, long wavelength disturbances and a mean field approximation he came out with figures corresponding to a 2 K temperature flux travelling at 7 m s^{-1} leading to a disturbance propagating at 70 m s^{-1}, which corresponds to a five day rotation of the atmosphere (moving, correctly, in the retrograde sense). By comparison with this, the related model of Alan Plumb (Meteorological Office) was intimidatingly mathematical, though his numerical results looked as good as those of Hinch's model.

Jack Meadows (University of Leices-

ter) attempted to explain the evolution of the Venus atmosphere, comparing the process with the evolution of the Earth's atmosphere. The two seem greatly different today, chiefly because of the lack of water on Venus. Meadows pointed out that the difference could be explained in terms of a photo-dissociation process operating strongly on Venus to break water down (through interactions involving carbon dioxide) to oxygen and hydrogen, the latter escaping into space. But he said further that there is no need to invoke this process, and that he would be prepared to accept that Venus has had a predominately carbon dioxide atmosphere "from the start".

In this model, CO_2 is produced by the effect of heat on carbonates and silicates in the Venus rocks. Several reversible reactions involving these compounds include carbon dioxide as a product, and the amount of CO_2 present would then depend just on the temperature. Once a little atmosphere existed, the greenhouse effect would allow conditions very like those on Venus today to be established, given the boundary condition of the present output of the Sun. Since the Sun is slowly warming up, this suggests an increase in the density of the Venus atmosphere in future. The initial wisp of atmosphere needed to start the process could either come from a burst of volcanism, or slower outgassing.

Venus without its atmosphere must have been much the same as Mercury is today, and J. E. Guest (University College, London) described with the aid of Mariner 10 pictures the surface of Mercury and impact theories of the cratering. Much of this material formed something of a travelogue; for the mixed audience, the most interesting feature of the Mariner 10 pictures is that signs of compressional faulting indicate that the planet has shrunk by one or two kilometres since its formation.

The new studies of the inner planets support Runcorn's attempts to describe Mercury, Venus, Earth, Moon and Mars in one relatively simple model. If Mars and Mercury are both cratered like our Moon, then surely Earth and Venus have been through the same bombardment, providing a clue to events early in the development of the Solar System. If tectonic processes have occurred on Mars (and shrinkage on Mercury) geophysicists may gain a better insight into continental drift on Earth. And if satisfactory models of the Venus and Mars atmospheres are developed, this must help in understanding the workings of our own atmosphere.

As long ago as 1958, the RAS and RMetSoc held a joint meeting on planetary atmospheres. The way things are going, it certainly will not be a further 17 years before another tripartite meeting about the inner planets is held.

The sea as a chemical system

from Peter S. Liss

The Dahlem Workshop on the Nature of Seawater was held in Berlin on March 10-15, under the chairmanship of Professor E. D. Goldberg (Scripps Institution of Oceanography).

IN comparison with the solutions normally studied by pure chemists, seawater is a very complicated medium. Not only is its ionic strength high (~ 0.7 M), but the ions in solution represent virtually all the naturally occurring elements, with concentrations of different elements ranging over at least twelve orders of magnitude. Furthermore, many of the dissolved species are involved in biological processes and reactions at the air–sea and water–sediment interfaces. Pure and marine chemists met at the workshop to discuss techniques and concepts in chemistry which could deepen our understanding of seawater and of chemical reactions occurring in the marine environment.

The ionic strength and composition of seawater are very different from those of the aqueous media normally used for the determination of stability constants. Calculation of the effect of seawater yields only approximate values and for more accurate work, the discussion group which dealt with this topic concluded that it is much better to employ the ionic medium scale, that is, to use seawater (real or artificial) as the reference solution. A self-consistent set of conventions was proposed and its use will allow the development of uniform and thermodynamically rigorous data for seawater equilibria. The new approach has many important implications for marine chemistry, including the redefinition of pH and redox potential, and the use of reference solutions which closely match seawater in ionic composition for pH and ion-selective electrode measurements. It will obviate the need to use single ion activity coefficients and lead to phasing out of the scheme involving 'apparent' stability constants, which has served chemical oceanographers well for many years.

Sample collection is a perennial problem in seawater chemistry and is especially acute for the sea surface microlayer. Most techniques available at present involve dipping a screen or plate into the water to collect a layer approximately 200 μm thick. For the rapid collection of milligram quantities of material, consideration should be given to the technique of foam fractionation in which air or nitrogen is bubbled through the water to create a foam which, because of its large surface-to-volume ratio, will very efficiently scavenge surface-active molecules.

Less than 10% of the dissolved organic matter in seawater has been characterised and an important objective should be the identification of the remainder in representative samples from the deep ocean, above the thermocline and surface microlayer. Techniques are probably available for detailed identification, but these tend to be complex and expensive. Characterisation into broad classes of compounds and major functional groups is simpler and will yield much important information.

Our present knowledge of the particulate fraction in marine waters is rudimentary. For instance, in order to measure size distribution of particles the change in solution conductance due to the presence of the particles is often employed. However, using this method only part of the size spectrum can be examined at any one time and what is really measured is not the particles themselves but a complicated function of the relative conductivities of the particle and the solution. A much more promising technique is to use single-particle scattering counters. These devices, which use laser sources, have already been employed in studies of hydrosols and aerosols.

Though much is known about 'pure' solids (for example the role of electrical double layers, ion exchange reactions, particle–particle interactions), in the marine environment such properties are certainly very substantially altered because the particles have an organic coating. At present very little is known about the properties and composition of such layers, although it should be possible to use techniques such as microelectrophoresis and potentiometric titration to identify some of the major organic functional groups in the coatings, as well as in organic particles more generally.

The goal of the workshop was certainly achieved in a number of areas of seawater chemistry; reported here are only a few of the topics discussed. Where less progress was made it was generally due to the inability of the marine chemists to state the problems in a meaningful way, rather than any reluctance on the part of the chemists to get to grips with the complexity of the sea as a chemical system.

●

'review article

Form and function of cat retinal ganglion cells

W. R. Levick*

Recent explorations of the morphology of retinal neurones, combined with neurophysiological recordings have made it possible to link specific anatomical types with particular physiological classes. At the same time, the relatively complete anatomical mapping of the retina has revealed some bias in the sampling of neurones by electrophysiological techniques.

THE retina has always offered special opportunities to students of the nervous system. It is an extension of the brain conveniently located outside the skull and lining the inside of the back of the eyeball where it is accessible to exploration with a microelectrode under direct visual control. The ganglion cells, which are the retina's output neurones, are spread out as an essentially two-dimensional sheet and constitute the innermost cellular layer of the retina.

The ganglion cell dendritic tree provides the morphologist with a unique opportunity because it is confined to two major dimensions of spread. The pattern of a well stained dendritic tree can thus be directly determined in its entirety when the retina is mounted flat on a slide. The difficulties of assessing three-dimensional structures (thick sections, confusion from overlapping and obliquely running processes) are largely avoided.

The constraints which determine the form of the dendritic tree are not easily comprehended in a representative piece of nervous system but in the retina the sideways spread of the tree strongly conditions how much of the subjacent retinal image is accessible to a particular ganglion cell. There are considerable variations in the spread and mode of branching of different dendritic trees in the inner plexiform layer of the retina and so one is encouraged to expect important relations between structure and function in the spatial domain.

Morphology of retinal ganglion cells

A great deal was known about the appearance of ganglion cells long before their function became accessible to direct investigation. Cajal[1] made superlative analytical use of the Golgi method, which stains only a very small number of the cells actually present, but reveals their form in minute detail (Fig. 1). Cajal was keen to put forward a general morphological plan for all vertebrate retinae, based on certain characteristic forms of the cells. In the case of the ganglion cells, his classifications were based on: (1) the relative sizes of the cell-bodies (giant; middle-sized; small); (2) the number of layers in which the dendritic tree was arranged (single-layered; bilayered; multilayered; diffuse—that is, no obvious layering); (3) the particular strata of the inner plexiform layer to which the dendritic branches were distributed (first, second, . . . fifth); and (4) the density or sparseness of the dendritic branching pattern. Independent application of these four dimensions could lead to a very large number of morphologically distinct types of ganglion cell. According to Cajal, however, not all of

the possible types were observed in a 'typical' mammalian retina. Taking his descriptions literally, one may count up to 14 classes in the general case. In the cat, there could be still fewer classes because Brown and Major[2] cast doubt on the generality of Cajal's multistratified inner plexiform layer. They could distinguish only two sublayers.

The interesting recent development in the cat has been the substantial simplification of the morphological classification which has at the same time opened up the possibility of relating the form of ganglion cells to their function as revealed by neurophysiological studies on the responses of single cells. Boycott and Wässle[3] achieved their simplified classification by studying the cells in plan view, rather than on as Cajal had done, and arrived at a qualitative separation into three classes—alpha, beta and gamma cells. The discrimination was based on the recognition of differences in the form of the branching pattern of the dendrites.

Examples of alpha cells are shown in Fig. 2a, and b. They have rather sparsely branched dendrites going relatively straight out radially from a large perikaryon. Figure 2c–e shows beta cells. At equivalent retinal locations they are smaller in all respects than the alpha cells and have more branches per unit area of dendritic field. Most of the gamma cells (Fig. 2f, g) are characterised by small, often oval, cell bodies and a few very thin dendrites branching much less frequently than alpha and beta cells. Gamma cells may be a less homogeneous population than the other two classes. The example of Fig. 2g illustrates a form which may be sufficiently distinctive to constitute a separate morphological type, the delta cells.

Information on the size of axons is important for comparisons with physiological data. Unfortunately, quantitative measurements of diameters are unsatisfactory because of irregular distortions along their length. Qualitatively, Boycott and Wässle[3] noted that at equivalent retinal locations the axons of alpha cells were larger than those of beta cells and these in turn were larger than those of gamma cells.

A key result from the analysis of Boycott and Wässle[3] was the demonstration of a systematic quantitative gradation in size of alpha cells and beta cells with distance from the centre of the area centralis. The area centralis in the cat is the analogue of the human fovea which corresponds with the centre of gaze. The gradation involved principally the diameter of the field covered by the dendritic tree and to a more limited extent the diameter of the cell body. The recognition that a characteristic morphology may have a systematic quantitative variation with eccentricity has been essential in side-stepping the impasse which would result from rigid adherence to fixed size ranges of cell bodies[4].

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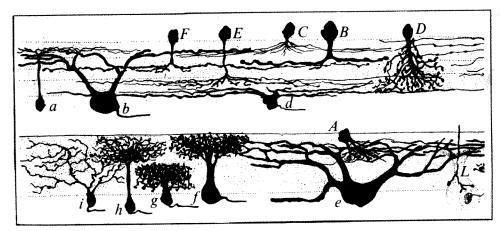
Fig. 1 Ganglion and amacrine cells from the dog stained by the Golgi method as seen in thick sections made perpendicular to the retinal layers. This is a selected part of Plate V in Cajal[1], who did not illustrate his work from the cat, but stated that cat, dog, pig and other mammals were virtually identical. Giant (*b*, *e*), middle-sized (*f*), and small (*a*, *d*, *i*, *h*, *g*) ganglion cells (all the rest are amacrine cells) have their dendritic trees running upwards and spreading out laterally in various fashions in this side-on view. It is interesting to compare *b* and *e* with alpha cells (Fig. 2 *a*,·*b*); *f*, *g*, *h* with beta cells (Fig. 2 *c*, *d*, *e*); *a*, *d* with gamma cell (Fig. 2 *f*) and *i* with a delta cell (Fig. 2 *g*), but Cajal unfortunately did not provide scales or relate the cells to eccentricity from the area centralis.

Physiological types of retinal ganglion cells

Cat ganglion cells became acecssible to functional testing by the technique introduced by Granit and Svaetichin[5]. The principle was to record the train of impulses which is the output message of a ganglion cell by bringing a delicate, insulated probe very close to the cell body. Rushton[6] showed neatly that such early recordings represented the activity of individual 'giant' ganglion cells. It was impractical to study the spatial sensitivity of ganglion cells until Talbot and Kuffler[7] devised a cannulation method for introducing the microelectrode into the intact eyeball, thus preserving the natural imagery. With the application of fine, sharp, electrolyte-filled micropipettes[8] or tungsten-in-glass electrodes[9] which penetrate rather than rest on the surface of the retina it has become possible to record from a wide range of ganglion cells.

A key property of ganglion cells is the concentric organisation of their receptive fields. Kuffler[10,11] showed that an individual ganglion cell was sensitive over a substantial patch of retina (0.8–2 mm), rather as Hartline[12] had found much earlier in the frog. The cat, however, differed from the frog in that the type of responsiveness was not the same throughout the patch. If a ganglion cell responded to a small flashing test spot by a discharge at turn-on for locations near the centre, then the discharge would come at turn-off when the spot was located in an annular surround zone at some distance from the centre. Such a cell would be of the on-centre off-surround type. Off-centre on-surround fields were also found and the two types occurred in about equal numbers.

Classification of cat receptive fields

For many years it was accepted that there were only the two classes of ganglion cell (on-centre off-surround and off-centre on-surround) with a continuum of variation of centre sizes and a preponderance of small-centred cells in the area centralis[13]. Enroth-Cugell and Robson[14], however, showed that the population of concentric receptive fields could be dichotomised according to the nature of the spatial summation of excitation over the receptive field. In one class of cell the processes of spatial summation were linear (X cells) whereas in the other the processes were very nonlinear (Y cells).

Two other dichotomising subsidivisions of concentric receptive fields have recently been developed. The sustained–transient[9] or tonic–phasic[15] dichotomy was based on applying a battery of visual tests, one of which depended on the extent to which the response of a ganglion cell was sustained when the eliciting stimulus was kept steadily on the centre. The brisk–sluggish dichotomy[16] was based on the responsiveness of cells.

Brisk cells, which were encountered about six times as frequently as sluggish, could be induced to generate powerful responses by strong stimulation. The responses of sluggish cells increased far less steeply with stimulus strength and remained relatively feeble regardless of the form or conditions of stimulation.
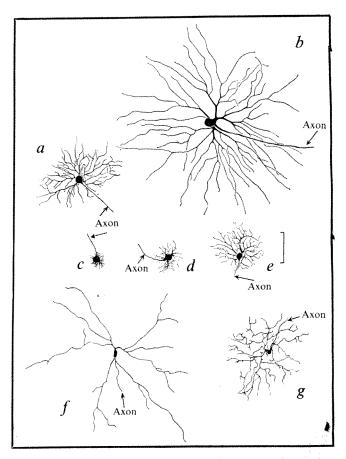
Fig. 2 Golgi-stained ganglion cells from flat-mounts of the cat's retina as seen in plan view (from Boycott and Wässle[3], with permission of the authors and the Physiological Society). *a*, *b*: Alpha cells. *c*, *d*, *e*: Beta cells. *f*: Gamma cell. *g*: Delta cell. The cells *a*, *c*, *f* were located at 1.2 mm from the centre of the area centralis, *d*, *g* at 2.9 and 2.8 mm and *b*, *e* at 10.0 mm. All are shown at the same magnification (calibration bar near *e* = 100 μm).

Table 1 Characteristics of five non-concentric classes

Receptive field types	Morphological identity
1. Concentric centre-surround types (comprising on-centre and off-centre varieties)	
a Brisk	
(i) Transient	Alpha
(ii) Sustained	Beta
b Sluggish	
(i) Transient	
(ii) Sustained	
2. Non-concentric	Gamma
a Local edge-detector	(delta, ...)
b Direction-selective	
c Colour-coded	
d Uniformity-detector	
e Edge-inhibitory off-centre	

Chiefly on the basis of the nature of responses when fine striped patterns moved over the receptive fields, it was argued that brisk-sustained cells were X cells (linear) and brisk-transient cells were Y cells (nonlinear)[16]. However, these affinities have not yet been checked with the specific stimuli used by Enroth-Cugell and Robson[14] in the case of sluggish-sustained and sluggish-transient cells. There is a recent tendency to classify cells as X or Y according to the conduction velocity of their axons. This is regrettable because the original principle of classification is interesting in its own right, and we do not know that it coincides with the conduction velocity principle for all types of cell.

Finally, it has gradually emerged[17-19] that there are rarely encountered ganglion cells having receptive fields organised quite differently from the concentric centre-surround type described by Kuffler. The situation has now been studied systematically on a large sample[20] and the characteristics of five distinct non-concentric classes described (Table 1). It was suggested[19,21] that such cells be referred to collectively as 'W cells'. The terminology has, however, become confused since Stone and Fukuda[22] now include sluggish concentric centre-surround receptive fields among the W cells, mainly on the ground that they have slowly conducting axons like the non-concentric classes.

Physiology and morphology

The diversity of types of ganglion cells immediately opens the possibility of relating morphological and physiological classes. One approach is to compare the spreads of dendritic trees and the sizes of receptive fields, just as Lettvin *et al.*[23] attempted many years ago in the frog.
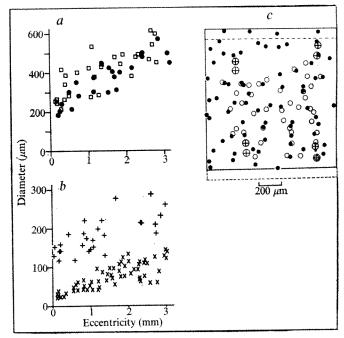
The first complication is that the cat receptive fields are composite. Should the comparison be with the total receptive field or only with the central subdivision? Gallego[24] pointed out that dendritic fields of giant ganglion cells were much smaller than total receptive fields measured by Kuffler[11]. Gallego[25], Brown and Major[2] and Dowling and Boycott[26] (monkey) argued that the correspondence should be between the dendritic fields and the centre components of receptive fields. It was not until systematic measurements of the extents of receptive field surrounds were made[27] that these arguments became more acceptable. It turned out that all but the largest dendritic trees are smaller than the smallest (≈ 400 μm in diameter) total receptive fields. The comparison should therefore focus on dendritic trees and receptive field centres.

The next complication is to decide what is involved in a measurement of centre size since there are systematic discrepancies in the data presented by different investigators[13-16,22,27-29]. Scattered light[11] and optical degradation of retinal imagery eliminate the possibility of stimulating individual receptors in isolation in order to determine those which contribute to the centre response. The external test stimulus can be made sharp and tiny but the retinal image cannot. So the experimenter must fall back on stimulus spots of significant size and more or

less indirect methods. Cleland and Levick[16] pointed out that any particular measurement convention implies some assumption about the physiological processes underlying the organisation of receptive fields. There are important qualitative differences in properties between the classes and therefore differences in the nature of the underlying processes. Since these are only partly understood[28], one must proceed empirically and not be put off by minor discrepancies. Moreover, one should not necessarily expect to find exact dimensional matching between receptive field centres and dendritic fields. Ganglion cells do not receive input directly from receptors but only through intermediate neurones (bipolar cells, amacrine cells) which themselves have significant lateral spread of their processes.

Two of the physiological classes are compact and dimensionally distinct from each other: the brisk-transient and brisk-sustained classes[16]. So are two of the morphological classes: the alpha cells and beta cells[3]. It is natural to attempt to pair these off. The centre sizes of brisk-transient units are large and match very well the dendritic fields of alpha cells (Fig. 3*a*), both in the trend with eccentricity as well as in the range of variation at particular eccentricities. The possible identification of these entities is supported by the fact that the axons of brisk-transient units had the shortest antidromic conduction times[16]. This implies that they had the largest axons as was observed for alpha cells[3].

The identification of brisk-transient units and alpha cells was confirmed more directly by mapping out the centres of

Fig. 3 Comparison of diameters of receptive field centres and dendritic fields of: *a*, brisk-transient units (□) and α cells (●); *b*, brisk-sustained units (+) and β cells (×) (dendritic field data from Boycott and Wässle[3], receptive field data from Cleland and Levick[16] with permission of authors and the Physiological Society). The centre sizes were obtained from maps of the receptive fields made with small flashed test spots and show close agreement in *a*. *b*, Brisk-sustained centres have the same gradient of size (and similar variation)with eccentricity as beta dendritic fields, but are systematically about 100 μm larger at each eccentricity (note the increased vertical scale). *c*, Comparison of the plotted positions of the geometric centres of the receptive fields of all the brisk-transient units (○) in a small patch of retina with the position of the cell bodies of all the alpha cells (●) in the same piece of retina after mounting and staining with cresyl violet. The four pairs of cross within circle symbols indicate the position of electrolytic lesions made at predetermined sites during recording and later identified histologically. The separation of the members of each pair indicates that a small downward shift of the receptive field map relative to the alpha cell map was required for optimal registration of centres with cells.

the receptive fields of all the brisk-transient cells throughout a small patch of retina. This was identified by lesions and stained with cresyl violet to show the cell bodies of all the ganglion cells present. The distinctive large ones are the alpha cells[30] and they agreed in number and position (Fig. 3c) with the brisk-transient receptive fields[31] to within experimental tolerance. In retrospect, this demonstration supports the identification of dendritic fields with the central component of the receptive fields in the case of the alpha cells.

The comparison[16] of brisk-sustained centres and beta dendritic trees[3] (Fig. 3b) is not quite so encouraging. Even though the diameters of both increase gradually with increasing eccentricity, the centres are systematically larger by about 100 μm. The comparable data of Stone and Fukuda[22] on centre sizes would provide a better fit, but it should be noted that they used an unusual convention for determining centre size. Support for the identification comes from consideration of the axons: brisk-sustained cells had intermediate antidromic conduction times[16] and beta cells had axons of intermediate size[3].

By exclusion the remaining physiological classes (concentric receptive fields of the sluggish kind and the rarely encountered non-concentric receptive fields) may be associated with the morphological gamma (and delta cells.) On both sides there is substantial heterogeneity and little to be gained from dimensional comparisons at the present stage. About the only common feature is the possession of a thin axon and slowly conducted impulses.

Relationship of physiological and histological results

The linking of functional and morphological entities opens up a realm of interrelated data in which the physiological and histological methods play complementary roles. For example, it is comparatively simple to trace the central path of the axon of a ganglion cell physiologically by strategic placement of stimulating electrodes in the brain. Tracing particular axons is a rather difficult feat histologically. On the other hand, it would be an Herculean task to enumerate the brisk–transient cells in the retina by exhaustive physiological exploration, yet it is comparatively simple to count them as alpha cells in a cresyl violet stained flat-mount. There were 6,212 in one whole cat retina[30].

Again, microelectrode recordings have long been suspected of giving rather selective access to a heterogeneous neuronal population. For example, brisk-transient receptive fields were encountered on about 10% of recordings in the area centralis and on more than 50% in the periphery[16], yet the histology showed alpha cells to be an approximately constant 3% of the total ganglion cell density throughout the retina[30]. They are greatly over-represented in physiological recordings. This selectivity, which may be related in some way with the cross sectional area of the cell body, is much harder to assess in the three-dimensional structure of the brain[32]. The result in the retina is a warning against uncritical acceptance of recording percentages of different classes of neurones as direct indicators of actual numerical preponderance.

As a further example of the interplay of the two approaches, the histology gives us the average density of alpha cells over small regions throughout the retina together with the corresponding dendritic field size. The physiology links the latter to the receptive field centre size which is the significant component for representing the visual field. It is then a simple matter to calculate the extent to which a given point in the visual field is redundantly represented by the set of brisk-transient cells. The answer was a relatively constant, 4.0 to 7.3 in spite of variation of cell density from about 200 mm^{-2} at the centre of the area centralis to less than 10 mm^{-2} at the far periphery. The increase in centre size with eccentricity is

thus sufficient to offset substantially the reduction in density[31] and suggests the existence of a regulatory process during development.

The interpretation of dendritic architecture remains a challenging problem. The issue is this. Do the branching pattern and shape of the dendritic tree have a special significance for combining the input signals to form the output train of impulses[23]? Or does the form of the tree merely reflect the growth pressures and competition confronting the ganglion cell in maintaining contact with the elements delivering the input signals? Undoubtedly, the type of synaptic connections made by the ganglion cell strongly determines the message that it transmits[33]. The discussions of Dowling and Boycott[26], Dowling[34] and Boycott[35] show how far interpretations may be taken without explicitly considering the branching patterns of the various neurones. Ganglion cells may receive input from bipolar cells both directly (dyad synapses) and indirectly (conventional synapses) through amacrine cells. In addition amacrine cells feed back on to bipolar cells (reciprocal synapses) and also make connections with other amacrine cells. The idea is that all of the rich variety of ganglion cell classes is assignable to varying proportions and types of direct and indirect inputs. The pattern of the ganglion cell dendritic tree may therefore be inconsequential for function apart from merely reflecting the spatial extent of connections.

Nevertheless, it is still possible to consider that a particular branching pattern coupled with a strategic geometric distribution of synaptic connections might be responsible for a more complex operation on the incoming excitatory and inhibitory signals than simple, weighted, linear combination. It has been argued with substantial experimental support[36] that inhibitory inputs on the lateral dendrite of the Mauthner cell of the goldfish have a greater attenuating effect on excitatory inputs entering far out on the dendrite than those impinging close in to the cell body.

If there is processing significance in dendritic form then the pattern of the alpha cell should be an early candidate for finer examination since its physiological counterpart, the brisk-transient class has strongly nonlinear summation properties[14].

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articles

Trilobite eyes and the optics of Des Cartes and Huygens

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The thick lenses in the aggregate eyes of a group of trilobites were doublet structures designed to eliminate spherical aberration. The shape of the optically correcting interface is in accord with constructions by Des Cartes and Huygens and is dictated by a fundamental law of physics. Trilobites may have evolved such sophisticated eye-lenses to maximise optic neurone response in a dimly lit environment.
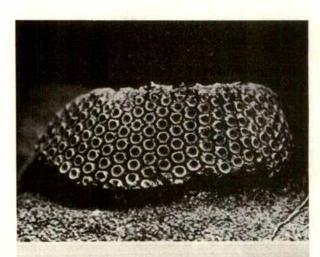
TRILOBITES, which occur in rocks ranging from Lower Cambrian (600 Myr old) to Upper Permian (250 Myr old), have the most ancient visual system known. From the beginning of their fossil record, compound eyes are present, symmetrically paired on the sides of the head. These compound eyes are primary structures and blindness in trilobites, which is not infrequent, is invariably secondary.

The primordial or holochroal eye, first found in the earliest genera, and thereafter taking many forms has numerous closely-packed lenses or prisms, each represented by a single crystal of calcite with its *c*-axis usually directed normal to the visual surface[1]. Most trilobites had such eyes, the main exception being the schizochroal eye, which is confined to the Ordovician–Devonian suborder Phacopina.

Schizochroal eyes, which are the subject of this study, have large thick biconvex lenses, each separated from its neighbours by a sclera; material similar in composition and structure to the rest of the cuticle. Eyes of this kind are present in the earliest Phacopina and there is some evidence that they were derived from the eyes of holochroal ancestors by paedomorphosis.

The structure of schizochroal eyes has been described in several publications[2,3] and it is now clear that in all known cases the lenses are doublets each of which has an upper unit of oriented calcite[4] (with its *c*-axis normal to the visual surface) interlocking with a proximally located intralensar bowl, the composition of which has not been determined. The distinction between these two units is apparent both in specimens which are preserved in limestone and thus retain their original structure, and in those preserved in silt or mudstone and subsequently decalcified. In the former case a compositional difference between the bowl and the upper lens unit is clearly visible in thin sections or polished surfaces unless diagenetic processes have destroyed or altered the primary structure; this is usually marked by a pronounced colour change. Invariably the lenses within any one eye are identical in structure. Where specimens are decalcified, the upper lens unit disappears, but the shape of its base may be preserved on the internal mould of the fossil. This is because the bowl has become detached or has decayed shortly after the death of the trilobite, and its former space is filled with silty or sandy matrix, preserving exactly the shape of the upper unit–bowl interface. The shape of this interface is clear

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in both kinds of preservation; there seem to be two basic kinds of structure with a range of intermediates between. The two fundamental kinds of lens structures were first described by Clarkson[3] from Ordovician Phacopina preserved as decalcified siltstone moulds. In *Dalmanitina socialis* (Barrande) from the Caradocian (Middle Ordovician) of Bohemia, the intralensar bowl is thin and indented centrally by a small dimple (Fig. 1a). Really well preserved specimens (RSM Geol. 1967-32; FMNH
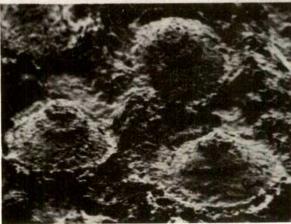
Fig. 1 *Dalmanitina socialis* (Barrande), RSM-Geol—1967–32 Letná Formation, Veselá. Bohemia. Caradocian (Ordovician). *a*, Left eye of a decalcified internal mould (steinkern) showing the intralensar bowls with central dimples. Slightly whitened with magnesium oxide (× 11). *b*, Latex replica of the eye surface of the same specimen showing the central dimple of each intralensar bowl in positive relief. Scanning electron micrograph (× 110).
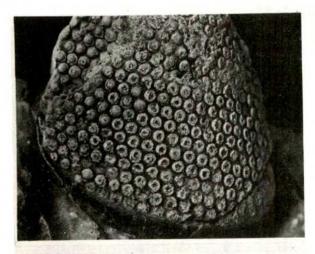
Fig. 2 *a, Dalmanites pratteni* Roy, FMNH P 16704, Devonian, Devil's Backbone, near Grand Tower, Jackson County, Illinois. Left eye of decalcified internal mould (steinkern) with intralensar bowls, partially filled with matrix (×4). *b, Zeliszkella lapeyrei* (Bureau), Gr. I. 40192. Llandeilian. (Ordovician). Traveusot-en-Guichen near Rennes, France. Part of the left eye of a decalcified internal mould showing an intralensar bowl in each lens cavity. Scanning electron micrograph (×110).

UC–1707) are rare, but from these, latex replicas can be made showing, as an average over many lens units, the surface shape as in Fig. 1*b*. The original reconstruction of Clarkson[3] has been slightly modified after a re-examination of the same material and some new material: the bowl is deeper and the upper surface is slightly more hyperbolic. Such lenses occur in other Dalmanitidae also.

The other kind of structure originally described from the species *Crozonaspis struvei* Henry[3], from the Llandeilian (Middle Ordovician) of Brittany, is perhaps better seen in the eye of *Dalmanites pratteni* Roy[5] from the Devonian of Illinois (Fig. 2*a*) and in *Zeliszkella lapeyrei* (Bureau) also from the Llandeilian of Brittany (Fig. 2*b*). Here the lens is highly convex with a large and thick intralensar bowl, indented not by a small dimple, but by a wide and hemispherical depression. Lenses of this kind are not only found in these species of Dalmanitidae, but are the norm in the Siluro-Devonian family Phacopidae, especially in the genera *Phacops* and *Reedops* in which the lenses of the eye are very large, highly biconvex and relatively few in number[6].

The example shown in Fig. 3 is a lens in horizontal section of a large-eyed Silurian *Dalmanites*. Dark field illumination shows the difference between the intralensar bowl and the upper unit. The latter has typical calcite cleavages showing it to be a single crystal (with the *c*-axis aligned along the lens axis); these stop short at the edge of the bowl. The shape of the bowl's upper surface is intermediate between the two end types mentioned above.

Corrected optics in phacopid lenses

We first discussed the possible function of schizochroal lens structure at the NATO sponsored Trilobite and Merostome Conference in Oslo in July of 1973. Subsequently, Levi-Setti recognised that the two basic patterns of the intermediate refracting surfaces in *Dalmanitina* and *Crozonaspis* approximated in shape to that of the aplanatic surfaces described respectively by Des Cartes[7] and Huygens[8].

Such generally aspheric refracting surfaces belong to the class of so-called Cartesian Ovals. They were originally designed to obtain lenses free of spherical aberration. The original constructions by Des Cartes and Huygens are reproduced in Fig. 4*a* and 4*c* respectively, where for comparison, the structure[3] of the lenses of *Dalmanitina socialis* (Fig. 4*b*) and *Crozonaspis struvei* (Fig. 4*d*) are also shown. The similarity between the shape of the upper unit in *Dalmanitina* and the Des Cartes construction, as well as in that of the upper unit in *Crozonaspis* and the Huygens construction is strikingly apparent.

Cartesian Ovals
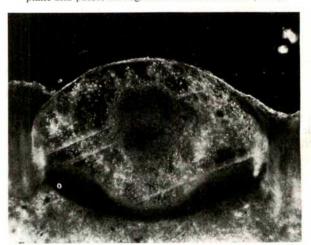
The physical principles underlying the function of the ovals of Des Cartes as optical refracting interfaces are derived from regarding these surfaces as the geometric representation of the stigmatic condition for an optical system (the requirement that a point in object space be mapped on to a point in image space). Their analytic expression, which is in general that of a fourth degree surface in Cartesian coordinates, takes a particularly significant form when cast in bipolar coordinates[9]. If the distances of a point *P* from two fixed points *O* and *O'* are called *r* and *r'* respectively, then *r* and *r'* are called bipolar coordinates of *P*. An equation connecting *r* and *r'* defining a locus for *P*, is called a bipolar equation. The ovals of Des Cartes are defined by the bipolar equation:

$$mr \pm nr' = k \tag{1}$$

where *m*, *n* and *k* are constants. If we now consider a system of two conjugate points *O* and *O'* in object (refractive index n_1) and image (refractive index n_2) space respectively, and *r* and *r'* the distances traversed by a light ray going from *O* to *O'* through a point *P* on the surface separating the two media, we can write the stigmatic condition as:

$$n_1 r + n_2 r' = c \tag{2}$$

Fig. 3 *Dalmanites* sp. Silurian. Locality unknown. Single lens in thin section in dark field illumination, showing the dark intralensar bowl, and calcite cleavages truncated by a diagenetically altered central mass. Diagenetic effects within other lenses of the same eye are very variable; in some lenses it is only incipient, whereas in others irregular masses of amorphous calcite have filled up much of the interior. This section is cut in the horizontal plane and passes through the centre of the lens (×70).
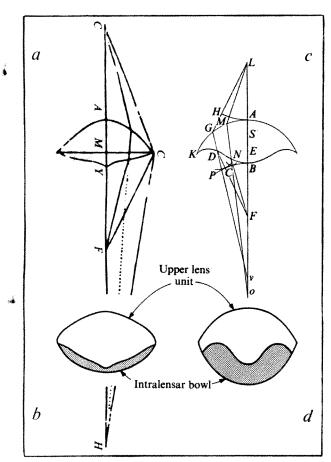
Fig. 4 *a*, Original construction by Des Cartes of an aplanatic lens in air, making use of two Cartesian Ovals. *b*, Reconstructed lens of *Dalmanitina socialis*. *c*, Original construction by Huygens of aplanatic lens in air making use of spherical first surface, and a Cartesian second surface. *d*, Reconstructed lens of *Crozonaspis struvei*[3].

with *c* as a constant. This equation implies that whatever the position of the point *P* on the interface of the two media, the optical paths (represented by the left hand side of equation (2)) between the conjugate points *O* and *O'* must be the same. Alternatively, the shape of the interface must be such that light will traverse the path from *O* to *O'* in equal times, either travelling along the straight line connecting the two points (the shortest path hence the shortest time) or any other off-axis path. In his construction of a lens free of spherical aberration, Huygens[8] adhered explicitly to this condition. Ultimately, equation (2) is a specialisation of Fermat's principle of least times, which states that no matter to what kind of reflection or refraction a ray is subjected, it travels from one point to another in such a way as to minimise the time taken. We recognise that equation (1), restricting to the + sign, and equation (2) have identical forms. The bipolar representation of the ovals of Des Cartes thus acquires an immediate physical meaning through fulfilling the stigmatic condition when describing the shape of an optical interface. In this sense the ovals of Des Cartes represent the most natural economical solution to the problem of constructing optical elements free of spherical aberration. In our derivation, the only physics involved consists of Fermat's principle: Snell's (or Des Cartes') law of refraction is in fact already an implicit consequence of the former, as first demonstrated by Huygens on the basis of the wave theory of light. The ovals of Des Cartes could of course be described more laboriously with recourse only to the (weaker) refraction laws[7].

Optical function of phacopid lens doublets

Were it not for the presence of the intralensar bowl in the trilobite lenses, there would be virtually a one-to-one correspondence in

shape and function between trilobite lenses and the optimal construction of Des Cartes and Huygens (allowing for differences arising from the fact the trilobite lenses operate in water rather than in air).

The function of the intralensar bowl has been investigated empirically by two approaches: first, graphical ray-tracing through the lens structure for example, that of *Crozonaspis*, and second, constructing a large-scale model of a *Crozonaspis* lens.

In the problem at hand we assume that the refractive indices of the media in contact with the entrance surface (seawater, *n* = 1.33) and the exit surface (body fluid, *n* = 1.34) are known. We also assume that the refractive index along the axis of the upper lens unit is equal to that along the *c*-axis of calcite (*n* = 1.66).

Given the interface profile actually observed, the unknowns are the refractive index of the intralensar bowl and the focal distance of the lens doublet as a whole. In principle the problem is completely determined by making use of the two constraining conditions: the stigmatic condition as an extension of equation (2); and the requirement of continuity of the light rays at each refracting surface. (It should be noted that since equation (2) satisfies Fermat's principle, the law of refraction does not represent an independent constraint.)

Using both approaches, by trial and error, we have determined that the intralensar bowl of *Crozonaspis* should have a refractive index of about 1.63, and the resulting focal length equals approximately one lens thickness measured from the exit surface. The *f*/number (inverse of the relative aperture) of this lens, operating in water is ~ *f*/1.1.

Given the shape of the upper unit as observed in *Crozonaspis* the intralensar bowl is thus seen as a necessary element to ensure convergence of an incident parallel beam of light to a sharp focus, when the lens is immersed in water. The intermediate Cartesian interface acts as a correcting surface, to make the doublet aplanatic.

The graphical ray tracing construction for the corrected lens versus a similar uncorrected one is shown in Fig. 5*a*, where the effect of spherical aberration for the latter case can be appreciated. The experimental verification of the function of the *Crozonaspis* lens is illustrated in Fig. 5*b*, which shows the focusing of a parallel beam of white light by a large scale model of the lens. Considering the uncertainties inherent in this reconstruction of the profile of the lens and the imperfections in the manufacture of the model, the focal plane of the corrected lens is remarkably well defined. The value of refractive index derived for the intralensar bowl may give a clue to its unknown chemical composition. Calcite was probably present, possibly together with organic material such as chitin.

Advantages and disadvantages

The fact that this remarkable lens doublet system functions in the manner empirically determined, provides independent evidence for the presence of oriented calcite[4] in the upper unit *in vivo*. It can, moreover, easily be appreciated that such a system is adapted for light collection and for the formation of a sharp and undistorted image in its focal plane.

Calcite is the basic structural component of the trilobite exoskeleton[10], but its orientation in the lenses is optimised to the function being performed. In the first place, this orientation is the direction along which the ordinary and extraordinary rays of calcite propagate with equal velocity, thus eliminating birefringence for paraxial rays. In the second place, along the *c*-axis the refractive index is maximal (*n* = 1.66), thus yielding the largest possible relative aperture (and thus optimising light-gathering) for a lens of the shape given.

The production of a good quality image, however, may not have been the main advantage to the trilobite of evolving such lenses. Because it is (to a first approximation) free of spherical aberration, the distribution of light intensity along the axis peaks more sharply in the region of the focal plane than it would in
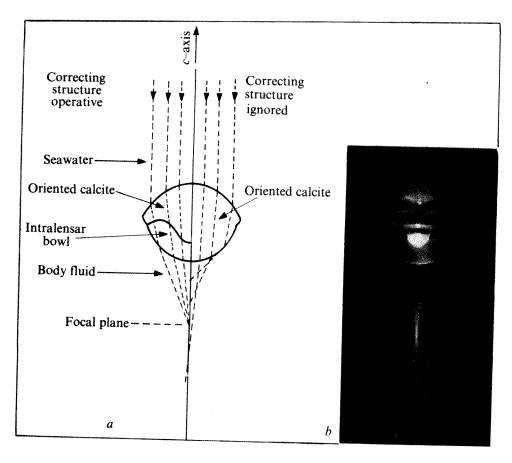
Fig. 5 *a*, Ray tracing through lens of *Crozonaspis struvei*. On left of the axis, the correspond structure is assumed operative indices of refraction: seawater 1. oriented calcite of upper unit 1. intralensar bowl 1.63, body fl 1.34. On the right of the axis, lens is assumed to have no inter structure, and made of orien calcite *n* = 1.66. *b*, Parallel be of white light incident upon a la scale model of a *Crozonaspis* le immersed in water, where the up unit is made of oriented calc (*n* = 1.66), and the intralens bowl is made of polysulpho (*n* = 1.63). The light beam is ma visible by a small amount of mi mixed with the water tank. T combination of refractive indic represent an optimum conditio arrived at after testing with intr lensar bowls made of vario plastics.

an uncorrected lens. A measure of this improvement is the full width at half maximum of such distribution. Scaled down to the actual size of the *Crozonaspis* lens (250 μm), we estimate the above width at about 20 μm, and possibly less. If the lenses were uncorrected, the corresponding width would approximate to about 100 μm. The increased concentration of light in the focal plane could conceivably raise the level of illumination in a dimly lit environment above the neural response threshold at the retina.

The use of a doubly refracting mineral such as calcite to construct a lens may seem disadvantageous. This drawback has been shown to be minimised by the orientation of the *c*-axis to coincide with the optic axis of the lens. Only for an object placed considerably off-axis would a secondary (extraordinary) image be formed, at varying depths, depending on the angle of incidence, but always deeper than the ordinary (o ray) image. As a consequence, the e ray image will not coincide with that formed by the o ray, nor will it focus in the same plane. One could envisage several means by which this ghost image could have been eliminated, although in any case it may have been of little consequence. A retina of limited thickness located in the image plane of the o ray would automatically filter out the e ray image: although accommodation of the eye would require a movable retina, a remarkable depth of field would still be available even with a fixed one. From the optical parameters of phacopid lenses, and requiring a circle of confusion in the retinal plane of diameter as small as 1 μm (the resolution limit due to diffraction), the depth of field would extend from several mm (~1 trilobite length) to infinity. Alternatively, one could invoke the use of photoreceptors sensitive only to the direction of polarisation of the o ray, thus eliminating the effect of the e ray image (the polarisation analysing capability of the rhabdomes is well known).

Finally, one could object that chromatic aberration may have offset the correction of spherical aberration. This is unlikely, however, since the environment under the sea is essentially monochromatic even at moderate depths.

Visual function of schizochroal eye

The function of schizochroal eyes can be better appreciated whei contrasted with that of holochroal eyes. Holochroal eyes ii trilobites normally have pronounced curvature in both the azimuthal and vertical planes (often assuming a toroidal shape and have numerous optical elements which could, as in insec and crustacean eyes, have formed a true visual mosaic. Schizo chroal eyes, however, have too few optical units to form a detailed mosaic image. Furthermore, the arrangement of the lenses in files often widely separated and pointing in diverging directions, leads to a discontinuous coverage of the object space[2] Such eyes as these were formerly thought[2] to be capable only of gross movement perception, though the discovery of corrected lenses now calls this view into question.

What use did phacopid trilobites actually make of their corrected lenses? They may have been used primarily as light concentrators, as has recently been proposed[11] for *Limulus polyphemus*. Alternatively, they may have served a more complex function. If the photoreceptor had the form of a thin retina, as suggested by the aplanatic properties of the lens, and if it was composed of numerous subunits, then the corrected image formed by the lens at the level of the focal plane or immediately beneath it, could be perceived as a micromosaic. The schizochroal eye could then be regarded more as an aggregate of individual eyes, each surveying a different part of the object space, rather than as a true compound eye. Evolution would have in this case proceeded from the external mosaic scheme of the ordinary compound eye towards the utilisation of an internal mosaic, much as in the lens–retina eye system of more advanced life forms. It is unfortunate that the genetic information of such a perfected visual apparatus became lost to further evolution in the animal kingdom, when the phacopid trilobites became extinct.

The presence of crystallised *in vivo* calcite in the lenses of fossilised trilobite specimens may prove valuable in a different context. It is conceivable in fact, that this primary mineral retained its original isotopic composition, in equilibrium with

the depositional environment. If so, trilobite lenses may afford an unprecedented opportunity for determining the temperatures of Palaeozoic seas using the $^{18}O/^{16}O$ relative abundance method[12].

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Evolutionary implications of different types of microbial enzymology for L-tyrosine biosynthesis

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Several patterns of enzymology for L-tyrosine biosynthesis exist in modern microorganisms, each differing in the apparent degree of regulatory efficiency. The extent of pathway evolution in a particular organism may reflect the relative selective pressure for regulation encountered in different ecological niches.

THE biochemical similarities of major metabolic pathways utilised in nature have reinforced a long standing concept of the biochemical unity of all forms of life[1,2]. Against this background, instances of diversity offer intriguing prospects for insight into evolutionary relationships. Biosynthetic pathways for amino acids are undoubtedly ancient and generally exemplify the concept of biochemical unity. The occasional existence of different amino acid-forming pathways seems to reflect significant phylogenetic divergencies, for example, biosynthetic pathways for L-lysine[3], L-methionine[4,5] and L-tyrosine[6,7].

Several enzyme arrangements that serve L-tyrosine biosynthesis in various microorganisms differ in pattern of regulatory control. At one extreme, species of blue-green bacteria (algae) possess a seemingly primitive pathway (pretyrosine) which has a less refined regulation, in contrast with that of the 4-hydroxyphenylpyruvate pathway of *Escherichia coli*. We report here results obtained with several other microorganisms in which features of pathway enzymology and regulation seem intermediate between those of blue-green bacteria and enteric bacteria. These data, considered together with previous published results, suggest an overall picture of pathway evolution in which modern organisms possess pathways representing different stages of an apparent evolutionary sequence. We propose that the selective pressure for regulation of L-tyrosine biosynthesis varies substantially within the ecological niche occupied by a given microbial group, and that the evolutionary stage of pathway development seen in modern organisms reflects the degree of selective pressure for regulation.

Pathways of L-tyrosine biosynthesis

The biosynthetic pathway for aromatic amino acids begins with the condensation of erythrose-4-phosphate and phosphoenolpyruvate, the first of seven enzyme reactions that culminate

with the formation of chorismate[8]. Chorismate is either used for tryptophan synthesis or converted to prephenate. So far as is known, all organisms able to synthesise L-tyrosine and L-phenylalanine utilise prephenate as the last common intermediate of a branching pathway. Although the enzyme sequence of the biosynthetic branchlet leading to L-phenylalanine may be universal, different L-tyrosine pathways exist. The pretyrosine and 4-hydroxyphenylpyruvate pathways, named for the intermediate between prephenate and L-tyrosine, are illustrated in Fig. 1. In each case both transamination and decarboxylation–dehydrogenation reactions occur—but in reverse order. The enzymes of each pathway are quite specific in organisms such as *Bacillus subtilis* and *Agmenellum quadruplicatum*[6,7], the most studied species exemplifying the 4-hydroxyphenylpyruvate and pretyrosine pathways, respectively. Hence, in *B. subtilis* a partially purified dehydrogenase utilises prephenate (prephenate dehydrogenase) but is not reactive with pretyrosine. No other dehydrogenase able to utilise pretyrosine was found in crude extracts or in fractions obtained during purification procedures. An aromatic transaminase of *B. subtilis* that was reactive with phenylpyruvate did not utilise prephenate[7]. Attempts to resolve other transaminases that might be reactive with prephenate were unsuccessful.

In *A. quadruplicatum* a single dehydrogenase is specific for pretyrosine, and no prephenate dehydrogenase activity was detected[6,7]. A single transaminase would seem to catalyse the transamination step within each pathway branchlet (that is, prephenate transaminase and phenylpyruvate transaminase). Transamination occurs far better with prephenate or phenylpyruvate than with 4-hydroxyphenylpyruvate[7]; likewise, in the non-biosynthetic direction L-phenylalanine is a far better amino donor than is L-tyrosine[6]. So far, the exclusive presence of enzymes of the pretyrosine pathway are known only in blue-green bacteria. The 4-hydroxyphenylpyruvate pathway for L-tyrosine synthesis seems to be the most widely distributed (Table 1), based on the comparative survey of dehydrogenase specificities in a limited range of microorganisms[7].

In addition to the pretyrosine and 4-hydroxyphenylpyruvate pathways, a third type of enzymology exists (denoted ambiguous in Table 1). Here transaminase and/or dehydrogenase activities are markedly ambiguous in substrate recognition. For example, *Pseudomonas aeruginosa* has the enzymological potential to synthesise L-tyrosine by either pathway. Table 2 shows the
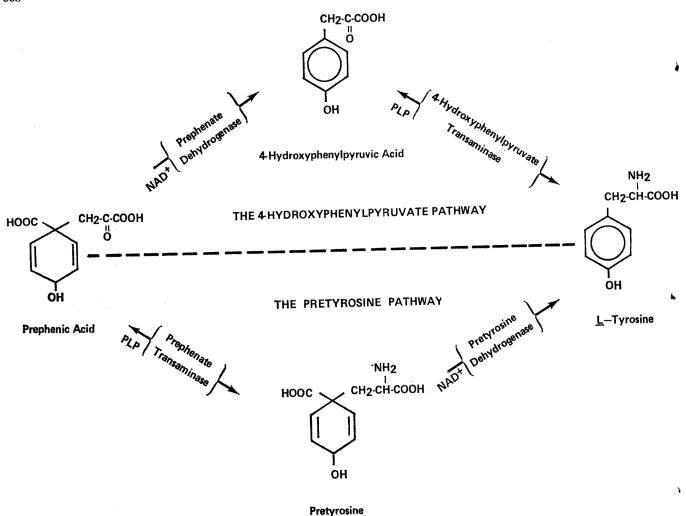
Fig. 1 Enzyme sequences of L-tyrosine biosynthesis. The upper sequence is the 4-hydroxyphenylpyruvate pathway, and the lower sequence is the pretyrosine pathway. A third ambiguous type refers to the presence of ambiguous transaminase (prephenate/4-hydroxyphenylpyruvate) and dehydrogenase (prephenate/pretyrosine) activities that allow either sequence to operate. NAD^+, nicotinamide adenine dinucleotide; PLP, pyridoxal-5'-phosphate.

substrate ambiguities observed for enzymes of *Neurospora crassa* and *P. aeruginosa*. In *N. crassa* only prephenate-specific dehydrogenase activity was found. A nonspecific transaminase, however, utilises either prephenate or 4-hydroxyphenylpyruvate. We have found (unpublished observations with S. L. Stenmark-Cox) that transaminase reactivity with prephenate in *N. crassa* can be expressed *in vivo*, resulting in dead-end accumulation of pretyrosine. Prephenate dehydratase mutants of *N. crassa* accumulate prephenate which is readily transaminated to pretyrosine, and substantial amounts of pretyrosine are accumulated in the growth medium. In *P. aeruginosa* an apparent single species of dehydrogenase is recovered[9] that reacts with either prephenate or pretyrosine. Although several transaminase activities were resolved from crude extracts of *P. aeruginosa* (I, II and III of Table 2), each transaminase species exhibited similar substrate ambiguity with respect to prephenate and 4-hydroxyphenylpyruvate utilisation.

Regulation of three pathway types

Three general patterns of L-tyrosine biosynthesis are discernable, exemplified by *A. quadruplicatum* (pretyrosine), *P. aeruginosa* (ambiguous) and *B. subtilis* (4-hydroxyphenylpyruvate). Each organism possesses a distinctive pattern of regulation for L-tyrosine synthesis, as compared schematically in Fig. 2. Unlike the L-tyrosine branchlet, the enzyme sequence within the L-phenylalanine branchlet is invarient in all species studied (that is, prephenate dehydratase followed by phenylpyruvate transaminase). Inevitably, prephenate dehydratase has been

found to be feedback inhibited efficiently by L-phenylalanine (Fig. 2, bottom row).

In *B. subtilis* (Fig. 2, right panel), the tyrosine and phenylalanine sequences are symmetrically analogous, each containing an initial irreversible reaction followed by a transamination step

Table 1 Pathways of L-tyrosine biosynthesis

Taxonomic occurrence	Type of enzymology	Apparent efficiency of regulation
Blue-green bacteria	Pretyrosine	+
Pseudomonas	Ambiguous [D], [T]	+ +
Neurospora	Ambiguous [T]	
Aerobacter		
Bacillus		
Brevibacterium		
Clostridium	4-Hydroxyphenylpyruvate	+ + +
Escherichia		
Serratia		
*Phaseolus**		
Saccharomyces		

Data supporting this summary are given or cited in refs 6 and 7. The most detailed published data to document *Pseudomonas* and *Neurospora* enzymology patterns are given in Table 2. Details of pretyrosine enzymology in *A. quadruplicatum* and references documenting the distribution of pretyrosine, 4-hydroxyphenylpyruvate or ambiguous pathways in nature are given in refs 6 and 7. [D], [T] Means that both dehydrogenase and transaminase activities exhibit substrate ambiguities, whereas [T] indicates that only transaminase (prephenate/4-hydroxyphenylpyruvate) is ambiguous in substrate recognition. These pathway types are illustrated in Fig. 4.
* Our recent results with cotyledon tissues from species of *Phaseoli* reveal very substantial pretyrosine pathway enzyme activities.

Table 2 Multi-substrate reactivities of L-tyrosine biosynthetic enzymes

Microorganism	Dehydrogenase substrates				Transaminase substrates	
	Prephenate	Pretyrosine			Prephenate	4-Hydroxyphenylpyruvate
			$(\text{nmol min}^{-1}\text{ mg}^{-1})$			
N crassa	8 2	0			6 4	10 3
P aeruginosa	24 6	32 4		I	32 9	131 8
				II	56 9	89 8
				III	124 8	274 8

Mycelia of a wild-type strain of *N crassa* (74-OR 23-1A) were collected after 72 h growth at 25 °C in Vogel's minimal medium[23] supplemented with 2% (w/v) sucrose Cultures were started from heavy conidial inoculations Mycelial yields from 10 l of medium were lyophilised Extracts were prepared by addition of mycelial powder to 50 mM potassium phosphate buffer, *p*H 7 5, containing 50 mM KCl, 2mM MgCl₂ and 20% (w/v) sucrose The suspension was stirred at 4 °C for 20 min and centrifuged at 25,000*g* for 30 min The clarified supernatant was dialysed at 4 °C against 500 volumes of buffer overnight This cell-free extract was used for dehydrogenase and transaminase assays In *P aeruginosa* partially purified enzyme preparations were used The dehydrogenase and transaminase I were recovered from DEAE cellulose as before[9] Transaminase II and III are the leading and trailing bands of activity eluted from hydroxylapatite[9] Details of the dehydrogenase assays were previously given[9,10] The validity of NADH formation as a measure of pretyrosine dehydrogenase activity was verified by the fluorometric measurement of tyrosine[24] The following reaction mixture was used to measure transaminase activities 50 µl of 40 mM glutamate, 100 µl of 5 mM prephenate or 4-hydroxyphenylpyruvate, and 50 µl of enzyme preparation After 20 min of reaction at 37 °C, the volume of the reaction mixture was brought to 5 ml with 2 5 N NaOH When prephenate was used as substrate, 100 µl of 1 N HCl was added and the incubation was continued for 10 min prior to the addition of NaOH to convert prephenate to phenylpyruvate Phenylpyruvate absorbance was measured at 320 nm and 4-hydroxyphenylpyruvate absorbance was measured at 331 nm, using a Gilford spectrophotometer

Regulation of each pathway is classically straightforward Excess L-tyrosine feedback inhibits prephenate dehydrogenase (Fig 2, top right), while excess L-phenylalanine feedback inhibits prephenate dehydratase (bottom right) In *E coli* the pathway and its regulation is essentially identical to that of *B subtilis* with the exception that prephenate dehydratase and prephenate dehydrogenase are both multifunctional proteins, each also catalysing the chorismate mutase reaction[11]

Unlike *B subtilis*, the pretyrosine pathway of *A quadruplicatum* contains an initial reversible step, activity of which is not affected by L-tyrosine A degree of control is indirectly accomplished through the activating effect of L-tyrosine on prephenate dehydratase[6] Thus, high intracellular levels of L-tyrosine would tend to favour flow of metabolites towards L-phenylalanine and away from L-tyrosine (Fig 2, top left) The apparent K_m for prephenate of the competing prephenate dehydratase (0 23 mM) and prephenate transaminase (1 05 mM) activities suggest that flow towards phenylalanine is intrinsically favoured The transaminations of phenylpyruvate and prephenate are probably catalysed by a single protein[6], this has the same affinity (K_m, 1 05 mM) for prephenate or phenylpyruvate but has a five-times greater V_{max} with phenylpyruvate The conditions assumed at the top left of Fig 2 (excess tyrosine, limiting phenylalanine) would promote a relative increase in the intracellular supply of phenylpyruvate together with a decreased supply of prephenate (owing to enhanced prephenate dehydratase activity) This would favour transaminase function as phenylpyruvate transaminase (rather than prephenate transaminase) On the other hand, high intracellular levels of L-phenylalanine would tend to shift prephenate flow towards L-tyrosine formation (Fig 2, bottom left) Inhibition of prephenate dehydratase would increase levels of prephenate substrate for transamination while decreasing levels of phenylpyruvate substrate for transamination

The regulatory pattern for L-tyrosine biosynthesis in *P aeruginosa* can be viewed as intermediate between those of *A quadruplicatum* and *B subtilis* To whatever extent the pretyrosine reaction sequence may be realised *in vivo* owing to substrate ambiguity in *P aeruginosa*, the overall picture of regulation (or lack of it) would resemble that of *A quadruplicatum* The middle panel of Fig 2, however, depicts the probable domination *in vivo* of the 4-hydroxyphenylpyruvate sequence in *P aeruginosa*[7] Prephenate is preferentially channelled towards L-phenylalanine by virtue of a single, multi-functional protein[12] which catalyses both the chorismate mutase and prephenate dehydratase reactions Prephenate dehydrogenase, which is subject to relatively weak competitive inhibition by L-tyrosine, has been characterised recently[9] When L-phenylalanine is present in excess (Fig 2, bottom middle) inhibition of the second half-reaction of the enzyme complex leaves the prephenate formed in the first reaction free for utilisation by

prephenate dehydrogenase[12] The pseudomonad pattern of regulation resembles *B subtilis* in its feedback regulation of the initial reaction of the tyrosine branchlet On the other hand, the pseudomonad enzymological pattern resembles that of blue-green bacteria in the preferential flow of chorismate toward L-phenylalanine and in the dominating regulatory role of L-phenylalanine, directly in the phenylalanine branchlet and indirectly in the tyrosine branchlet[12] Preferential entry of prephenate into the L-phenylalanine branchlet has been observed in several other microorganisms[13,14]

Possible ancestral pathway

It is reasonable to assume that the most ancient biochemical pathways were unregulated, for regulatory properties seem to be secondary refinements that must have followed the primary acquisition of catalytic capabilities Branching amino acid pathways such as those forming aromatic amino acids and branched-chain amino acids probably originated as simple, uncomplicated pathways forming a single amino acid Subsequent additions of pathway branchlets resulted in families of related amino acids Certain abiotically-formed amino acids in the primitive *milieu* inevitably became growth-limiting before others Biosynthesis of a limiting aromatic amino acid would have conferred a selective advantage on organisms with this capability If one assumes that the tryptophan sequence (or any other chorismate-containing pathway, for example, to folate or ubiquinone) originated before the phenylalanine and tyrosine pathways, tyrosine and phenylalanine-forming enzymes may have evolved as shown in Fig 3, a sequence which is based on non-enzymatic chemical reactivities of chorismate and prephenate Tiny amounts of L-phenylalanine and pretyrosine could have been synthesised from chorismate in ancient cells without the benefit of new enzymes Chorismate is readily rearranged thermally to form prephenate[15], and this conversion can be substantial at physiological temperatures Prephenate, in turn, is quite labile at acid *p*H, and even mildly acidic conditions consistent with cell growth can promote acid-catalysed conversion to phenylpyruvate[16] Assuming that some ancient transaminase possessed the broad specificity that commonly characterises modern transaminase enzymes, the dual possibility existed for transamination of phenylpyruvate to L-phenylalanine and/or of prephenate to pretyrosine Like prephenate, pretyrosine is acid-labile[6] and would readily be converted to L-phenylalanine

Subsequent selective advantages arising from the biosynthesis of L-phenylalanine would favour the evolutionary development of enzymes to catalyse the previously non-enzymatic steps more efficiently Pretyrosine may also have been a component of ancient proteins, and indeed the possibility that it may be an amino acid residue in modern proteins of blue-green bacteria has not yet been explored In any event, the availability of the
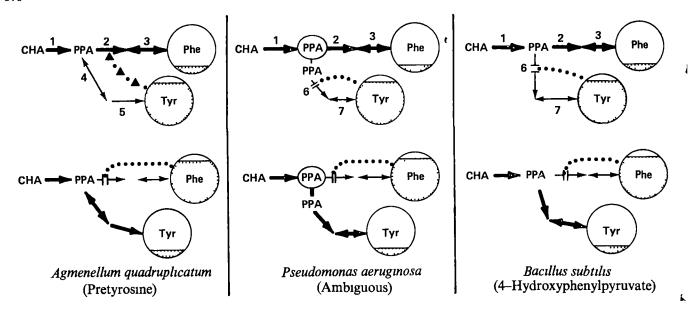
Agmenellum quadruplicatum
(Pretyrosine)

Pseudomonas aeruginosa
(Ambiguous)

Bacillus subtilis
(4-Hydroxyphenylpyruvate)

Fig 2   Regulatory patterns of L-tyrosine/L-phenylalanine biosynthesis in *A quadruplicatum* (left), *P aeruginosa* (middle), and *B subtilis* (right) The upper row of diagrams depicts an unbalanced nutritional state where L-tyrosine (Tyr) is available in excess while L-phenylalanine (Phe) is limiting At the other extreme (bottom row) L-phenylalanine is present in excess while L-tyrosine is limiting The flow pattern of intermediary metabolites is indicated by heavy arrows CHA, chorismate, PPA, prephenate In the middle panels the circled PPA denotes PPA that is channelled by the 1-2 enzyme complex[12], and which is not readily available to enzyme 6 = ●●●, feedback inhibition, ▲●▲, enzyme activation Enzyme notations 1, chorismate mutase, 2, prephenate dehydratase, 3, phenylpyruvate transaminase, 4, prephenate transaminase, 5, pretyrosine dehydrogenase, 6, prephenate dehydrogenase, 7, 4-hydroxyphenylpyruvate transaminase References documenting the regulatory effects shown have been detailed elsewhere for *A quadruplicatum*[6], *P aeruginosa*[9] [12] and *B subtilis*[10,27-29]

pretyrosine molecule would permit the development of L-tyrosine-synthesising capability following acquisition of a single new enzyme Presumably, this occurred by duplication of a gene encoding an ancient dehydrogenase, followed by mutational modification of that duplicated gene[17,18] to produce a pretyrosine-reactive dehydrogenase The hypothesis that L-phenylalanine biosynthesis preceded L-tyrosine biosynthesis is consistent with the uniformity of L-phenylalanine enzymology and regulation in nature (Fig 2)

Evolutionary alteration of specificities

The ultimate ancestral pathway for L-tyrosine biosynthesis that is hypothesised in Fig 3 is identical to that of modern blue-green bacteria In Fig 4, blue-green bacteria are depicted as having the most primitive pathway while *E coli* possesses one of the most evolved pathways of L-tyrosine biosynthesis Assuming that the 4-hydroxyphenylpyruvate pathway of modern *E coli* originated as a pretyrosine sequence, one can view the L-tyrosine pathways of other modern organisms as reflecting stages of pathway evolution that may once have been intermediate evolutionary stages in *E coli*

Beginning with the transaminase and dehydrogenase specificities found in modern blue-green bacteria, transaminase and dehydrogenase enzymes may have evolved through stages similar to those still retained in modern pseudomonads and *Neurospora*, finally developing the reversed specificity of enzymes found in the 4-hydroxyphenylpyruvate pathway of *E coli* Thus, in the stage exemplified by *Pseudomonas*, a single NAD-dependent dehydrogenase has evolved reactivity with prephenate and diminished reactivity with pretyrosine Likewise, a single transaminase possesses an increased reactivity with 4-hydroxyphenylpyruvate but less reactivity with prephenate Continued selection for this flip-flop change of specificity, resulting in loss of pretyrosine pathway capability and increased 4-hydroxyphenylpyruvate pathway capability, is shown in Fig 4 (lower right) The pathway of *N crassa* is shown as intermediate between pseudomonads and *E coli*, owing to the lack of ambiguity of dehydrogenase (as in *E coli*) but the
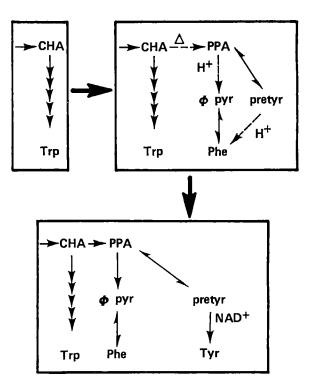
Fig 3   Hypothetical evolution of capability for L-tyrosine and L-phenylalanine biosynthesis in ancestral cells Enzymatic capability for synthesis of the aromatic amino acid family is postulated to have evolved in the order L-tryptophan, L-phenylalanine and L-tyrosine CHA, chorismate, PPA, prephenate, φ pyr, phenylpyruvate, pretyr, pretyrosine, Trp, L-tryptophan, Phe, L-phenylalanine, Tyr, L-tyrosine Solid arrows and dotted arrows represent enzymatic and non-enzymatic reactions, respectively Prephenate and pretyrosine are acid-labile, undergoing quantitative conversion to phenylpyruvate and L-phenylalanine respectively Chorismate is converted to prephenate at elevated temperature (͵)
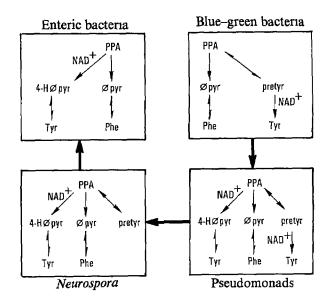
presence of prephenate/4-hydroxyphenylpyruvate transaminase (as in pseudomonads) Finally, *E coli* exhibits dehydrogenase and transaminase specificities that are distinct from those seen in blue-green bacteria

Pathway regulation and ecological niche

The 4-hydroxyphenylpyruvate pathway of *B subtilis* and many other eubacteria such as *E coli* seems to be more sophisticated (in terms of regulatory versatility) than the other L-tyrosine-forming pathways of various microorganisms This is not to suggest that *E coli*, for example, is biologically superior to other microorganisms in any general sense The presence of a more highly evolved pathway implies an evolutionary response to strong selective pressures It is clear that biological success in one ecological niche involves different selective pressures than in another We suggest that the regulatory efficiency of each microbial type of enzyme sequence for L-tyrosine synthesis is generally appropriate to the ecological niche in which a given microbe is successful At one extreme, *E coli* resides in the gut of man where the nutritional regimen is one of periodic feast and famine A selective advantage results from a simple, direct and efficient regulation of both enzyme activity and synthesis *E coli* is geared for substantial amino acid biosynthesis during famine as well as for efficient utilisation of exogenous amino acids during feast

The ecological relationships of blue-green bacteria are quite different The competitive advantage of blue-green bacteria in nature is their photoautotrophic mode of metabolism They utilise small organic molecules poorly, and heterotrophic capabilities are generally undeveloped In fact, amino acid molecules and other organic compounds are often inhibitory to the growth of autotrophic bacteria[19-21] In the ecological niche of blue-green bacteria, characterised by the constantly diluting effect of the fresh or marine water *milieu*, most L-tyrosine molecules probably originate from endogenous metabolism When exogenous tyrosine is present under laboratory conditions, the regulation seems poor since no direct mechanism exists to decrease L-tyrosine biosynthesis The regulatory scheme shown in Fig 2 may, however, satisfactorily partition the flow of endogenous prephenate into the tyrosine and phenylalanine branchlets If so, one must assume that the kinetic properties of the competing enzymes at the branchpoint are appropriate for some endogenous and relatively constant level of prephenate Thus, the primitive stage of development of the L-tyrosine pathway may be adequate for the ecological niche of blue-green bacteria The persistence of the pretyrosine pathway in modern blue-green organisms may reflect the lack of selective pressure for more elaborate regulation

Enteric and pseudomonad microorganisms provide an especially apt comparison of the relationship of metabolic specialisation and regulation The most striking metabolic capability of pseudomonad organisms is their versatile utilisation of organic compounds as sole sources of carbon, nitrogen and energy Enteric bacteria provide the most dramatic examples of repression control of biosynthetic enzyme levels, although scarcely any repression control of biosynthetic enzymes is known in pseudomonads On the other hand, pseudomonads provide the most impressive examples of induction control for numerous degradative enzymes Thus, metabolism seems to be geared to biosynthesis in enteric bacteria and to catabolism in pseudomonads Since survival value is undoubtedly equated with these metabolic capabilities, selection for the most finely tuned regulatory mechanisms may be maximised within the pathways providing metabolic specialisation The presence of enteric-like repression control mechanisms for biosynthetic enzymes in pseudomonads would not necessarily be an advantage, since that might impose restrictions on overall capability for regulatory variation of degradative enzymes In natural soil and water environments, substantial accumulation of free amino acids does not occur[22] Pseudomonads in nature do not experience the regular exposure to high levels of amino acids and so on which place a premium on responsiveness to end-product

Fig 4 The evolutionary development of the 4-hydroxyphenylpyruvate (4-H⌀pyr) pathway viewing the existing pathway in *E coli* as the most highly evolved and that in blue-green bacteria (algae) as the least evolved Abbreviations are as given in Fig 3

levels in enteric bacteria Even plant or animal pathogens (such as *P aeruginosa*) do not encounter the regularity of concentration flux, as is the case with enteric organisms It seems reasonable that selection for efficiency of regulation would be maximised for biosynthetic pathways in enteric microorganisms Presumably other organisms have different metabolic priorities that are dictated by differing selective pressures For example, rigorous selection for regulatory efficiency would be expected for catabolic enzymes of pseudomonads and for enzymes of autotrophic metabolism in blue-green bacteria

It is interesting that the apparent differences in regulatory refinement for enzymes of L-tyrosine synthesis are paralleled by the same qualitative differences in apparent regulatory efficiency for 3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase in *A quadruplicatum*[21], *P aeruginosa*[25] and *B subtilis* (or *E coli*)[26]

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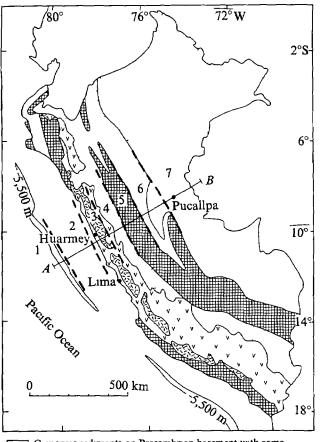29 Pierson, D L, and Jensen, R A, *J molec Biol*, 90, 563–580 (1974)

Vertical crustal movements of the Andes in Peru

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Although the Peruvian Andes provide no evidence of the widespread, strong deformation which might be expected in a tectonic belt widely held to overlie a zone of oceanic subduction, consideration of the tectonic and magmatic history shows that the occurrence of large scale, vertical block faulting in that region is not incompatible with a plate tectonic model

THE important association between vertical crustal movements and magmatism during the evolution of the Andean mountain range was recognised[1,2] in the nineteenth century Most mountain belts later became interpreted in the light of alpine tectonics[3] and comparisons with the Alps continued throughout the period during which mountain belts were viewed as expressions of the final phase of geosynclinal cycles The concept of plate tectonics eventually emphasised the fundamental differences between many mountain belts[4], but the Andes was equated[4] with the cordilleras of western North America where it was postulated that major doming of the continental margin above underthrust oceanic lithosphere has caused overthrusts on the continent and the spread of sediments over the adjacent oceanic crust There is little supporting evidence for this hypothesis in the Peruvian Andes[5,6], where consideration of the past 100 Myr of tectonic and magmatic history suggests a tectonic model which is different from that postulated[4] for western North America The description is based on detailed mapping of the western Andes between latitudes 10° and 10° 30' S (ref 7) and published descriptions of adjacent regions[8-10], supplemented by radiometric age determinations (P A Wilson, unpublished)

Regional tectonics

During Cretaceous time, northern Peru consisted of two ribbon-like belts of subsidence known as the West and East Peruvian troughs[8,11] (Fig 1) They were separated by a belt of relative uplift, the Marañon Geanticline[8], and a second belt of relative uplift, the Paracas Geanticline[12], formed the western edge of the continent Each belt was founded on a block of Precambrian basement with thin Phanerozoic cover[13], and was bounded by

Fig 1 Simplified geological map of Peru (after ref 13) showing the tectonic framework of northern Peru during Cretaceous and early Tertiary time 1, Oceanic crust, 2, Paracas Geanticline, 3 and 4, West Peruvian Trough (3, Paramonga Block, 4, Chavin Block), 5, Marañón Geanticline, 6, East Peruvian Trough, 7, Brazilian Shield *A-B,* line of sections of Figs 2, 3 and 5

Cainozoic sediments on Precambrian basement with some Palaeozoic and Mesozoic cover

Cretaceous–Tertiary Coastal Batholith

Cretaceous–Tertiary volcanics

Mesozoic shelf sediments

Precambrian basement and Palaeozoic cover

Fig 2 Schematic section (*A–B,* Fig 1) across Peru 100 Myr ago, showing subduction of oceanic crust (1), and the movement of major blocks of continental crust 2, Paracas Block, 3, Paramonga Block, 4, Chavin Block, 5, Marañon Block, 6, East Peruvian Trough block, 7, Brazilian Shield Casma Volcanics were erupted on the Paramonga Block from the rising Coastal Batholith (Patap Complex—black, tonalite—irregular dashes), continental crust—white, mantle—stippled, T, Tapacocha Axis
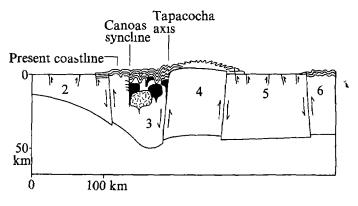
Fig. 3 Schematic section (part of *A–B,* Fig 1) showing the different kinds of deformation of Mesozoic sedimentary and volcanic rocks which resulted from uplift of different crustal blocks (numbered as on Fig 2) about 95 Myr ago Ornamentation as on Fig 2

steep fractures and shear zones Oscillatory vertical movements of these ribbon-like blocks controlled both the deposition of sediments and their near-surface deformation from at least Cretaceous to early Tertiary time

The West Peruvian Trough was itself divided into western and eastern belts by another steep shear zone, the Tapacocha Axis[14], which between 115 and 95 Myr ago allowed the western belt to subside about 4,000 m more than the eastern belt (Figs 1 and 2) The crustal blocks which underlie the western and

eastern belts of the West Peruvian Trough are here called the Paramonga and Chavin blocks, respectively, and those which underlie the Paracas and Marañón Geanticlines are called the Paracas and Marañón blocks, respectively (Fig 2)

Casma Volcanics and the Coastal Batholith

The Casma Group of volcanic rocks was erupted through the descending Paramonga Block from the rising Coastal Batholith below, forming a pile of andesite lavas, sills and pyroclastic rocks 3,000–6,000 m thick (Fig 2) Meanwhile, about 3,000 m of sandstone and limestone[10] accumulated on the more slowly subsiding Chavin Block Relative variations between the amount of volcanic material erupted and the rate of subsidence of the Paramonga Block resulted in oscillatory cycles of deeper and shallower marine and terrestrial volcanism within the Casma Group The age ranges of ammonites interbedded with the volcanic sequence indicate that most eruptions occurred in the late Middle Albian and early Upper Albian (about 100 Myr ago)[7,14] The occurrence of granodiorite, diorite and gabbro fragments in some pyroclastic flows suggests that the Casma Group of volcanic rocks was erupted through, and was probably associated with, plutonic rocks similar to the Coastal Batholith which was later emplaced into the Casma Group

The oldest intrusions of the Coastal Batholith are numerous bodies of hornblende gabbro and diorite, collectively called the Patap Complex[7], which were emplaced into the eastern side of the Paramonga Block (Fig 2) They contain more hornblende than pyroxene and seem to have crystallised from hydrous magmas Many bodies of hornblende gabbro show rhythmic layering which may have formed in association with volcanoes within the upper part of the Casma Group
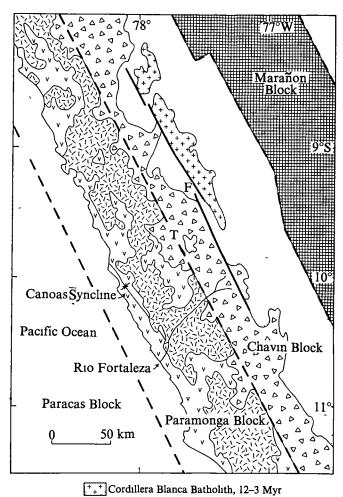
Regional deformation

Intermittent pulses of deformation during intrusion of the Patap Complex folded the Casma Group into folds with vertical axial surfaces and sub-horizontal fold axes parallel with the Andes The anticlines are mostly broad and open whereas the synclines are narrow and tight (Fig 3) The gentle northwesterly plunge of the Canoas Syncline reveals that at structurally deeper levels to the south-east, the fold becomes even tighter and passes into a 2–4 km wide belt of isoclinal folds The grade of metamorphism also increases progressively towards structurally deeper levels, from being hardly noticeable in the north-west where the structure is an open fold, to greenschist and amphibolite facies where the folds are isoclinal The Canoas Syncline lies near, but is slightly oblique to, the western edge of the Coastal Batholith (Fig 4), and a similar belt of strong deformation and syntectonic metamorphism occurs at the eastern edge of the batholith along the Tapacocha Axis (Fig 3) These belts of deformation may be the upward expression of major shear zones in the crust below, which partly controlled the edges of the rising batholith, and were channels of high heat flow from the rising plutons

The style of deformation of the contemporary rocks on the Chavin Block was completely different (Fig 3) There, the sequence of limestone and sandstone was folded as a single unit by décollement on the underlying Lower Cretaceous Oyon Shale in the south, and the Jurassic Chicama Shale in the north[9,10] Upright ribbon-like folds with axes traceable for 100 km or more occur on the centre of the Chavin Block, whereas towards the margins of the block, the folds are overturned outwards, and lower fold limbs are increasingly attenuated and pass into outwardly directed thrusts

To the east, the thin, Cretaceous sedimentary rocks and pre-Mesozoic basement of the Marañón Block were broken up by normal and reverse faults[9,10] (Fig 3)

The distinctions between the Paramonga, Chavin and Marañón blocks which had previously been marked by different kinds and thicknesses of sediments during differential rates of block subsidence, were thus emphasised by three distinct deformation styles during uplift of the blocks The Paramonga Block was underlain by rising magmas of the Coastal Batholith

Fig 4 Map of northern Peru showing the spatial association of the Coastal Batholith and Casma Volcanics, both restricted to the Paramonga Block, and the Calipuy Volcanics which spread on to the edge of the Chavin Block Map simplified after ref 15 T, Tapacocha Axis, F, fault

and the folds represent the high penetration of ductile deformation above steep, basement shear zones associated with the rise of the batholith through the block The Chavin Block to the east was not infiltrated by rising magma, and it rose intact and was tilted or arched, causing folding of the Cretaceous sediments which lay on it by gravity sliding on a lubricant of shale Further to the east on the Marañón Block, the shale lubricant
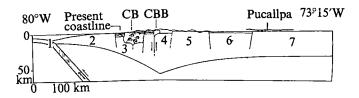

Fig 5 Schematic section (A–B, Fig 1) across Peru, showing the sum of Tertiary and Quaternary block movements, the location of the whole Coastal Batholith (CB) and Cordillera Blanca Batholith (CBB), and the present topography Continental crust—white, mantle—stippled Crustal blocks numbered as on Fig 2, thickness of continental crust after ref 16

was absent and faults of the rigid basement penetrated the thin cover of sedimentary rocks The overturned folds and out-wardly directed thrusts at the eastern edge of the Chavin Block locally transgressed onto the Marañón Block and were dissected by the block faulting (Fig 3) This suggests that the centre of the Chavin Block was raised higher than the Marañon Block, and that the folds flowed eastwards under the influence of gravity

Calipuy Volcanics and the main intrusions of the Coastal Batholith

A 2,000–4,000 m thick terrestrial sequence of andesite, dacite and rhyolite, lava and pyroclastic flows, called the Calipuy Volcanics, lies unconformably on folded Casma Volcanics on the Para-monga Block, and spreads eastwards over the non-volcanic Cretaceous sediments on the western part of the Chavin Block (Fig 4) It is intruded by many plutons of the Coastal Batholith and is probably the product of fissure and caldera eruptions from tonalite, granodiorite and granite magmas which formed much of the batholith

The batholith is a tabular complex of plutonic rocks, 50 km wide and about 15 km thick which extends for over 1,100 km along the eastern part of the Paramonga Block (Fig 4) It is made up of a large number of plutons of mainly tonalite, granodiorite and granite which were emplaced by repeated cauldron subsidence into the centre of the pile of Casma and Calipuy volcanic rocks to within 3 km or less of the surface[12] Individual plutons form ring dykes and rectangular bodies parallel with the continental margin, with steep walls and flat roofs and floors

Chronology of volcanic and plutonic history

The Casma Volcanics were erupted 100–95 Myr ago They immediately preceded, and locally overlapped in time with, the intrusion of the earliest, Patap Complex of the Coastal Batho-lith, and were probably derived entirely from magmas of the rising batholith[12] The Calipuy Volcanics which postdate uplift and erosion of the deformed Casma Volcanics and Patap Complex, were probably erupted from magmas which formed the younger complexes of the batholith 95–30 Myr ago (P A Wilson, unpublished) There was thus a close association between volcanic eruptions and plutonic intrusions in the eastern part of the Paramonga Block for at least 70 Myr during oscillatory vertical movements of the block

Uplift and erosion of the modern Andes

The succession of erosion surfaces which are preserved on the western flank of the Andes indicate sporadic uplift of a gently undulating landscape formed by erosion of the Coastal Batholith and its volcanic envelope[7] The erosion surfaces increase in altitude with increasing antiquity and distance from the western edge of the Paramonga Block The steady increase in the slope of the erosion surfaces with age indicates that uplift occurred mainly by arching of the Chavin Block and westward

tilting of the Paramonga Block Major uplift and erosion of the modern Andes occurred between the time of emplacement of the last major plutons of the Coastal Batholith 30 Myr ago (P A Wilson, unpublished) and the eruption of an ignimbrite down the aggraded ancestoral Rio Fortaleza Valley 6 Myr ago (P A Wilson, unpublished) Later incision of the valley through this ignimbrite indicates further uplift of at least 500 m within the past 6 Myr

In addition, the Chavin Block was split by a complex of steep major faults along the western foot of the Cordillera Blanca (Figs 4 and 5) The faults have been intermittently active from at least 6 Myr ago to the present time, and have raised the eastern part of the Chavin Block, which forms the crest of the Andes, a further 500–1,000 m The fault complex may be the upward expression of a deep fracture–shear zone system which was active during late Tertiary time and which localised the ascending magmas that formed the Cordillera Blanca Batholith 12–3 Myr ago[17]

Andean structure and plate tectonics

The Peruvian Andes do not show any evidence of the strong deformation of the continental margin which occurred[4] in western North America during eastward movement of oceanic crust beneath the continent In Peru the relative rigidity of the continent enabled it to float intact on the weaker oceanic crust which was deflected beneath it, and only weak tensile and compressive stresses were intermittently transmitted through the continental margin, causing oscillations of the crustal blocks The steep fault and shear zones which bound the main crustal blocks are parallel with similar structures which were active during late Palaeozoic and early Mesozoic time, and they may all have been initiated in the same major tensile stress regime which during Precambrian time split the South American con-tinent from continental crust to the west

Fieldwork was done during tenure of a National Environment Research Council postdoctoral research fellowship held at the University of Liverpool I thank P A Wilson for permission to quote unpublished K–Ar radiometric age determinations, and E J Cobbing, A E Escher, and A K Higgins for criticism of the manuscript

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letters to nature

Is Cir X-1 a runaway binary?

RUNAWAY stars are produced when there is a supernova explosion in a close binary system Usually such an explosion disrupts the binary system, but Gott[1] has suggested that in a significant fraction of such supernovae the collapsed residue of the explosion may be retained in orbit around the runaway

second star, the resultant system being a binary with high eccentricity and large space velocity We suggest here that Cir X-1 is such a system

Figure 1 shows a 408-MHz radio map of the Cir X-1 region The map was produced from observations made with the Molonglo Cross radio telescope, and is centred on the recently
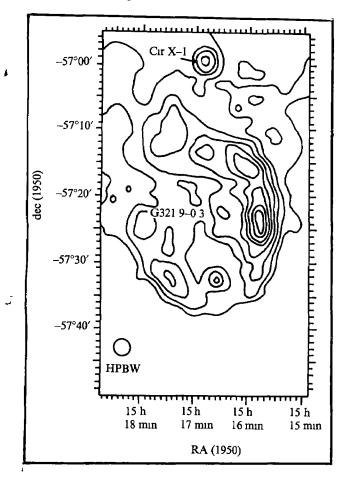
Fig. 1 Map of the supernova remnant G321 9—0 3 made at 408 MHz with the Molonglo radio telescope (half power beam-width, HPBW, as shown) The contour unit of brightness temperature averaged over the beam solid angle is 23 8 K (corresponding to 0 1 Jy for a point source)

discovered supernova remnant (SNR) G321 9—0 3 (refs 2, 3) Figure 1 also shows a point radio source approximately 7' north of the SNR boundary The position and flux density estimates for the point radio source are given in Table 1

The position of Cir X-1 has recently been revised by Jones *et al*[4] and the point radio source lies well within their 2' × 1' error box, approximately 16" from their best position We therefore suggest an association of the X-ray source Cir X-1 and the point radio source Location of the radio source within the error box is not sufficient grounds for a definite association The probability of finding a radio source of 0 5 Jy inside the X-ray error box is, however, approximately 1 in 3,000 (see ref 5) The detection of correlated short term variability in the X-ray and radio sources, as found in several other similar systems, would secure the identification, and we plan to make such observations in the near future

The radio flux density estimates give a spectral index (flux proportional to v^α) $\alpha^{5,000}_{480} = -0\ 25$ This is an unusual spectrum for a point radio source (singling out the source from most extragalactic sources or galactic H II regions[6]) The radio source associated with Cyg X-1 also has an unusual spectral index, with a non-thermal spectrum below about 2,700 MHz and a spectral upturn at higher frequencies[7] Between our measuring frequencies of 408 MHz and 5,000 MHz, a similar change of spectral index for the Cir X-1 radio source could explain the apparently shallow spectrum obtained Cir X-1 and Cyg X-1 do differ, however, in one important respect the ratio of radio to X-ray emission for Cir X-1 is at least an order of magnitude greater than that for Cyg X-1 An alternative explanation for the unusual radio spectral index obtained for Cir X-1 could be long

term variability of the radio source, since the 5,000 MHz observations were made 18 months later than those at 408 MHz, but such variability would in itself make the source of considerable interest A possible pulsar-like behaviour of the radio source has been investigated at Molonglo, without success (J Sutton, personal communication) A pulsar with period less than 160 ms, or a dispersion measure greater than 500, would not have been detected in this search

Are Cir X-1 and the SNR G321 9—0 3 related and, in particular, is Cir X-1 a runaway system ejected from the supernova? A distance estimate for the SNR may be inferred from comparison of its surface brightness and angular diameter with those SNRs with well determined distances Using such a technique, Clark and Caswell[8] derive the following parameters for G321 9—0 3 linear diameter 39 pc, distance from Sun 5 5 kpc, distance from galactic plane — 29 pc In spite of limitations in the technique, the uncertainty in these parameter estimates is believed to be less than ±30% The estimate of age for SNRs, however, remains particularly uncertain, a comparison of the surface brightness of G321 9—0 3 with the values for those few remnants of known age suggests a possible age range of 2 × 10⁴ to 10⁵ yr These parameters then give a required average transverse velocity for Cir X-1, if it were indeed created in the supernova, of 10²–10³ km s⁻¹, assuming the centroid of the radio remnant gives the position of the initial supernova explosion

The properties of Cir X-1 are in fact compatible with it being a runaway binary at a distance of ~5 kpc and with an age of ~ 10⁴–10⁵ yr, as shown below

Until an optical counterpart is discovered, the distance to Cir X-1 must remain uncertain, and difficult to estimate X-ray spectra of Cir X-1 are strongly attenuated[9,10] at energies ≤2 keV Below approximately 6 keV the spectra can be fitted with an exponential (thermal Bremsstrahlung) spectrum with a temperature in the range 3 5 × 10⁷–4 3 × 10⁷ K and a hydrogen column density 2 5 × 10²²–2 9 × 10²² cm⁻² It is likely that a considerable fraction of the attenuation is circumstellar, however, if we assume it is all interstellar we obtain an upper limit to the distance of 9 kpc taking an average interstellar density of 1 H atom cm⁻³

Jones *et al*[4] have reported that there is no star brighter than 15 mag in their error box By considering stars of various spectral types we can set a lower limit on the distance to the source by taking the average visual absorption to be 1 5 mag kpc⁻¹ for this direction in the galactic plane Five of the eight variable X-ray sources with known optical counterparts have been identified with binary systems having early type stars as primaries For a B0 supergiant, consistent with its not being brighter than 15 mag, a lower limit to the distance is 5 0 kpc, for an A0 supergiant the distance drops to 4 5 kpc and for a B0 main sequence to 4 0 kpc For later stars the minimum distance decreases accordingly

The distribution of 2–10 keV luminosities for galactic X-ray sources has been investigated by Margon and Ostriker[11] who found a range of values between 10³⁶ and 10³⁸ ergs⁻¹ with a peak near 5 × 10³⁷ ergs⁻¹ Taking the 2–10 keV Uhuru maximum count rate of 720 s⁻¹ (1 count s⁻¹ = 1 7 × 10¹¹ erg cm⁻² s⁻¹) gives a distance range of 1 to 9 kpc with the most likely distance, corresponding to the peak of the distribution, at 6 kpc We conclude that the data now available for Cir X-1 are not inconsistent with it being at the distance of the SNR (5 5 kpc) Further improvements in defining this distance are clearly needed, but must await an optical identification

By analogy with all of the other galactic variable X-ray sources Cir X-1 is likely to be a binary system, the X-ray production being due to accretion onto a collapsed object Although observations of X-ray 'eclipses' have been reported[4,12], no unique binary period has yet been found, Jones *et al*[4] claiming that if a binary period exists it must be greater than 12 d

If Cir X-1 originated in the supernova with remnant G321 9—0 3, it is likely that the explosion increased the eccentricity and the period of the binary system For a system with a large eccentricity and long period, the accretion process may break

	RA (1950)	dec (1950)	Flux density (Jy)
408 MHz (Molonglo)	15 h 16 min 47 3s	− 56° 59′44″	0 46
5,000 MHz (Parkes)	15 h 16 min 46 4s	− 56° 59′54″	0 25

The 408 MHz and 5,000 MHz positional estimates have 12″ and 20″ standard errors respectively

down for a time around apastron giving what seems to be an eclipse in the light curve and so hampering the determination of a binary period Such a determination would also be hampered if the system is precessing so that it does not seem to eclipse on each orbit It is likely that after some considerable time tidal forces will tend to circularise the orbit[13], as observed in such a system as Cen X-3 which is believed to be 10^5–10^6 yr old Without an identified period, it seems possible that Cir X-1 is a relatively young system, 10^4–10^5 yr old (the estimated age of the SNR), whose orbit has not been circularised as in the older, better understood binary systems This is the first possible SNR–binary system association to be discovered and gives credibility to theories suggesting that supernovae have given rise to all the close binary systems, if not all the galactic X-ray sources

We thank Professor R L F Boyd for encouragement, Dr J Sutton for providing unpublished data and Dr A Fabian for comments

Note added in proof An optical plate taken by A J Longmore and R D Cannon with the UK 48-inch Schmidt telescope at Siding Spring, Australia, shows that there are two stars brighter than 20 mag within 20″ and five stars within 30″ of the best 408-MHz position

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Acceleration of pulsars to high velocities by asymmetric radiation

Here we propose that pulsars are accelerated by the emission of asymmetric low frequency electromagnetic radiation and rapidly attain characteristic velocities exceeding 100 km s^{-1}

Pulsars are believed to be rotating neutron stars that have intense surface magnetic fields The structure of the field is often assumed to be dipolar, with the magnetic axis of symmetry

passing through the centre of the neutron star at an angle to the rotation axis Such a field is modelled nicely by a magnetic dipole of moment μ located at the centre and oblique to the axis of rotation An oblique dipole rotator generates electromagnetic radiation at the rotation frequency and the energy radiated is at the expense of the rotational kinetic energy[1] The rotation period consequently increases, in rough agreement with observation

Various sources of evidence suggest that the oblique dipole rotator is an oversimplified model Recent studies of magnetic variable stars[2,3] have shown that an inclined magnetic dipole moment, displaced from the rotation axis, provides an improved model for reproducing the surface field Displacements of 0 1 or more of a stellar radius are not unusual Of the six pulsars[4,5] known to have interpulses well separated from their main pulses, three (PSR0823+26, PSR0904+77, PSR1929+10) have weak interpulses located midway between their main pulses The magnetic dipole moment for these pulsars is perpendicular to the spin axis, and is possibly displaced from the centre in directions parallel and perpendicular to the spin axis If the pulsed luminosities are proportional to the square of the field intensity, then the displacements from the spin axis are approximately one-third of the radius of the neutron star The other three (PSR0531+21, PSR0950+08, PSR1055–51) have strong interpulses located asymmetrically at 140°–150° from their main pulses The field configurations of these pulsars are also easily reproduced by a dipole moment, in a plane perpendicular to the spin axis, and offset from the spin axis a distance that is again about one-third the radius of the neutron star It is of interest to note that Earth and Jupiter also have oblique off-centred dipole fields All known observations suggest that planetary and stellar objects are generally oblique off-centred dipole rotators

We consider, then, a pulsar with a magnetic dipole moment that is arbitrarily oriented and also displaced from the rotation axis In cylindrical coordinates the components of the dipole moment are μ_z, μ_ρ, μ_φ, where the z coordinate is along the rotation axis Unlike an oblique centred dipole rotator that radiates only at the rotation frequency Ω, the oblique off-centred dipole rotator radiates at all harmonics of Ω Moreover, the radiation field is not that of a pure dipole, but consists of a superposition of both magnetic and electric multipoles of all orders If s is the distance of the dipole from the rotation axis, then when $s\Omega/c \ll 1$, most of the energy is emitted at the fundamental frequency and the total low frequency luminosity is approximately

$$L = \frac{2\Omega^4}{3c^3}\left[\left(\mu_\rho{}^2+\mu_\varphi{}^2\right) + \frac{2}{5}\left(\frac{s\Omega}{c}\mu_z\right)^2\right] \qquad (1)$$

The first term on the right, enclosed in the parentheses is the power radiated by a simple dipole rotator, whereas the second term in parentheses represents the power radiated by the sum of the electric-dipole and magnetic-quadrupole fields

In addition to an increase in luminosity, we find that the luminosity averaged over a rotation period is asymmetric about the rotational equatorial plane This asymmetric emission is the result of interference of magnetic-dipole radiation fields with electric-dipole and magnetic-quadrupole radiation fields The asymmetric emission of low frequency electromagnetic radiation results in a net reaction force on the neutron star that is approximately

$$F = -2s\Omega^5\mu_z\mu_\varphi/15c^5 \qquad (2)$$

in the direction of Ω On writing $F = \varepsilon L/c$, we have

$$\varepsilon = -\frac{s\Omega\mu_z\mu_\varphi/c}{5(\mu_\rho{}^2 + \mu_\varphi{}^2) + 2(s\Omega\mu_z/c)^2} \qquad (3)$$

for the 'radiation asymmetry coefficient' We note that ε has a maximum value of 0 16 when $\mu_\rho = 0$, $\mu_\varphi = 0 63s\Omega\mu_z/c$ Also,

when a pulsar is young, $s\Omega/c$ may be a significant fraction of unity and therefore values of $\varepsilon\sim0\,1$ are quite possible

For a young and rapidly spinning pulsar the luminosity and radiation force are both large, and it is possible that the radiation force rapidly accelerates the whole pulsar to a high velocity The acceleration of the pulsar is

$$dV/dt = \varepsilon L/Mc \qquad (4)$$

and since εL is proportional to Ω^5, maximum acceleration occurs on the time scale $I\Omega_0^2/2L_0$, where I is the angular moment of inertia and the zero subscript denotes initial values Thus, the velocity of the pulsar, as a result of asymmetric emission of radiation, is

$$\Delta V \sim \varepsilon_0 I\Omega_0^2/2Mc \qquad (5)$$

For a neutron star of radius 10 km this gives $\Delta V\sim10^{-4}$ $\varepsilon_0\Omega_0^2$ km s^{-1}, and for initial values $\varepsilon_0\sim0\,1$, $\Omega_0\sim10^4$, the final velocity is $\sim10^3$ km s^{-1} It is evident that with reasonable values of ε_0 and Ω_0 this radiation acceleration mechanism is efficient and capable of explaining the remarkably high velocities of many pulsars For example[6], PSR113+16 has large proper motion that is equivalent to a velocity in excess of 380 km s^{-1}, if the pulsar is at a distance of 120 pc (In our analyses (to be published) we find evidence for pulsar velocities exceeding 700 km s^{-1})

Most pulsars are probably born in binary systems, and it can be shown that the mass ejected from close binary systems in a supernova event is generally inadequate to unbind the binary members[7] It can be shown, however, that the radiation reaction force is initially sufficiently strong to wrench apart all but the closest binaries, thus explaining why most pulsars are not members of binary systems In the case of those pulsars that survive as members of binary systems (for example, the Hulse–Taylor pulsar[8], PSR1913+16), the radiation force plays a dominant role in the evolution of the orbital parameters

The theory of radiative acceleration of pulsars with its various ramifications and implications will be presented elsewhere.

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Comets and interstellar masers

RECENT advances in our understanding of the origin of the Solar System have led to renewed speculation on the origin of comets[1,2] Oort[3] proposed that long-period comets originate in a cometary cloud about 10^5 AU from the Sun and Cameron[4] has suggested that this cloud has its origin in association with the primordial solar nebula Other investigators have placed the origin in the interstellar medium[5]

Whipple and Lecar[6] have suggested that long-period comets arise from the interaction of the primordial solar nebular with the interstellar medium The opposing action of the solar wind from the new star and the interstellar radiation field squeezes the excess nebular material into a ring at the nebular edge

where saturated molecules such as H_2O and heavy metal compounds condense

High density rings around young stellar and protostellar objects at heliocentric distance $r = 10^4$–10^5 AU have also been suggested as the location of interstellar masers[7] Because these objects contain large fractional abundances of H_2O and perhaps other saturated molecules such as CH_3OH, and exist in an environment believed to be favourable to comet formation, there may exist an ontological relationship between masers and comets I suggest that the long-period comets are among the condensed remnants of cooled interstellar masers formed in a dense region at the nebular boundary I shall discuss the dynamics of the maser–comet transition and suggest ways in which this relationship may be exploited to expand our understanding of star and planet formation

Maser radiation from OH, H_2O, SiO, and perhaps CH_3OH has been observed from small knots of gas and dust in the direction of molecular radio sources associated with H II regions, and also towards certain infrared stars[7-9] The most common source of maser radiation, the OH radical, is often associated with H_2O maser radiation and the H_2O maser is always closely associated with OH-maser sources[7] The sizes of the emitting regions vary from 10 AU for H_2O regions to 10^3 AU or greater for OH regions Theoretical considerations of the pumping mechanism place limits on the density of H_2 in these regions of $10^6\leqslant n(H_2)\leqslant10^9$ cm^{-3} (refs 7 and 10) Fractional abundances for OH of 10^{-6} and for H_2O of 10^{-5}–10^{-4} are suggested The H_2O region may be contiguous with the OH region but higher in density and temperature, and perhaps closer to the central star

It has been demonstrated (J C Weisheit, A Dalgarno and M O , unpublished) that the chemical process

$$O+H_2 \rightleftharpoons OH+H \qquad (1)$$

is the only source capable of providing the large abundance of OH H_2O is formed by

$$H_2+OH \rightleftharpoons H_2O+H \qquad (2)$$

and by ion–molecule reactions initiated by penetrating cosmic rays H_2O is removed slowly by the reverse reaction (2) and more rapidly by[11,12]

$$C^+ + H_2O \rightarrow HCO^+ + H \qquad (3)$$

and

$$H_2O + h\nu \rightarrow H + OH \qquad (4)$$

Other saturated molecules are removed by analogous processes Photons which ionise carbon and dissociate H_2O do not penetrate much beyond $10^{21}/n$ cm into a region with the normal interstellar dust-to-gas ratio The residual ionisation of C^+ from cosmic rays is small and, except at the edges of the maser region, most of the oxygen is bound into H_2O and other molecules for $T>200$ K The lifetime of H_2O is greater than 10^{12} s, except at the edges of the region, even if it lies near an O-type star Unsaturated chemical species are removed much more rapidly by reactions among themselves such as

$$OH+N \rightarrow NO+H \qquad (5)$$

$$OH+O \rightarrow O_2 +H \qquad (6)$$

with time scales of $10^{14}/n$ s (ref 11)

The elevated temperatures needed to produce masers are obtained in or near protostellar objects and young stars Observational evidence and theoretical considerations suggest that some masers exist in rings of hot dense gas on the edge of protostellar objects and nebulae associated with young stars[7] A shock front related to the infall of material into a new star or the interaction of the solar wind of a new T-Tauri star with the remnant nebular gas may generate maser action at several

points circumferential to the object Many maser point sources exhibiting variations on time scales of the order of a year are found associated with single compact H II regions which may be cocoon stars

Consider the thermal evolution of maser sources that lie on the edge of the nebula of a new star The chemical scheme and the theoretical excitation mechanism suggest $T \geqslant 300$ K in an OH–H_2O maser region[7] There is probably an excess of dust by comparison with the interstellar value because of accumulation at the nebular edge Carbon monoxide, initially present in substantial amounts in the interstellar medium (M O and A Dalgarno, unpublished), may be converted to CH_4 and other saturated molecules at the elevated temperatures of the maser Eventually, the shock wave that heats the region dissipates or moves on, and the maser cools Maser fluctuation periods and shock speeds indicate a lifetime for the hot phase of 10^8–10^{10} s Cooling proceeds through emission from grains and in molecular transitions A lower limit on the cooling rate is found from the cooling through thermalised CO transitions For the velocity gradients, temperatures, and densities typical of these regions, the cooling time is $10^3 n$ s (T de Jong, S-I Chu and A Dalgarno, unpublished) H_2O will itself cool the gas very efficiently Conversion of CO to hydrogenated species increases the cooling rate[13] Conceivably it is this conversion which shuts off the maser

The time scale for cooling is much shorter than that for chemical removal of the saturated species H_2O, NH_3, CH_4 and other saturated molecules are frozen in with their maser-region densities for $n \lesssim 10^9$ cm^{-3} at depths where ultraviolet photons do not penetrate On the other hand, OH, O, N and other chemical radicals disappear during cooling

The presence of H_2O and any other molecular hydrides increases the cooling rate in the maser region significantly as compared with the surrounding medium, even after maser action ceases The resulting pressure gradient and decrease in volume of the H_2O region may lead to a greatly enhanced density Simultaneously, the saturated species will accrete on to grains as they cool with the gas The time scale for accretion is $10^{17}/n$ s in the interstellar medium[14], and probably shorter in the outer regions of the nebula Thus the accretion of ice mantles of saturated species proceeds before they are chemically removed The combination of large pressure gradients and competing solar-wind and interstellar-photon pressure[6] may bring about the enhanced density of grains needed for accumulation of the matter into a comet[4] The mass of condensable elements in a cylindrical maser[7] of radius r_m and depth l is

$$10^{-3} \alpha \delta n M (\pi r_m{}^2 l) \qquad (7)$$

where M is the average molecular weight, δ the depletion factor and α the degree of conversion to condensable molecules for minor constituent elements In an H_2O-maser region[7] with $r_m = 5 \times 10^{13}$ cm, $l = 3 \times 10^{15}$ cm, $n = 10^6$ cm^{-3}, $M = 20$ and $\delta \sim \alpha = 1/3$, the condensed mass is about 6×10^{22} g, this corresponds to a comet nucleus of radius 200 km with density of 1 g cm^{-3}, reasonably close to the estimated values for bright comets within the uncertainties of the model For lower values of α and δ, or larger density (representing a different chemical evolution), a smaller comet is generated I have chosen a maser of relatively low density Masers of higher density may fragment into several comets The total mass of condensables available in a ring of density 10^6 cm^{-3} and width 10^{16} cm at a distance of 3×10^{17} cm from the primordial Sun corresponds to 10^9 large comets, enough to explain the observed frequency of appearance of new comets[2] This density is in accord with values derived for the maser region in the Orion nebula[10] at about 10^{17} cm from the HCN emission peak

If the postulated maser–comet relationship is correct, information on the origin and composition of comets can be obtained from observations of interstellar masers, and of masers from observations of comets The chemical imprint in comets of the early stages of the formation of the Solar System[18]

may yield information on the temperatures and densities at that epoch for the outer region of the nebula As an example, I shall consider the equilibrium involving CO, CH_4, H_2 and H_2O in comet and maser regions Donn[15] has suggested that this equilibrium may serve as an indicator of the temperature in the gas when the comet formed The overall reaction is

$$CO + 3H_2 + 59 \text{ kcalorie} \rightleftharpoons CH_4 + H_2O \qquad (6)$$

The forward direction is favoured over the reverse for $T < 1,000$ K (refs 16 and 17), but the rate-determining steps are much slower than the probable thermal time scales in a maser region None of the gas-phase reaction schemes proposed for the interstellar medium produce CH_4 in abundances comparable to CO[11,18] Observational and theoretical evidence (M O and A Dalgarno, unpublished) suggests that 10–100% of the carbon atoms are found in CO in dense interstellar clouds Therefore, unless CH_4 is produced efficiently on grain surfaces[14], one does not expect

$$n(CO)/n(CH_4) \ll 1$$

in interstellar clouds Since CH_4 is not produced in the cometary coma[19] that ratio should not be much different in comets unless the parent maser region has experienced a high temperature phase of evolution

Similarly, H_2O is clearly in substantial abundance in the maser region Since H_2O is not removed during cooling, the H_2O should contain a substantial fraction of the oxygen in the cometary coma Contrary evidence would suggest heating of the parent region to $T > 2,000$ K (ref 17), or penetration of that region by dissociating photons Interpretation of chemical imprints is complicated by the fact that comets originating on the outer edge of the maser ring, where photons penetrate, will have a different thermal and ionisation history from those formed deep within the ring In addition, observed masers may occur near stars with mass greater than $1 M_{\odot}$, whereas our comet observations are restricted to the solar neighbourhood In spite of these complications, the maser–comet relationship may be a useful tool in understanding the early Solar System

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Invalid 4.01-Gyr model U–Pb 'age' of the Nakhla meteorite

NAKHLA, an achondrite composed dominantly of the minerals 'diopside' and olivine[1], is possibly a unique meteorite It has a ^{39}Ar–^{40}Ar age of about 1 3 Gyr (refs 2 and 3) and recently its Rb–Sr age was shown by Papanastassiou and Wasserburg[4] to be similar In their discussion, these authors[4] use the concept

Table 1 U and Pb concentrations and Pb isotopic composition in samples of Nakhla

Sample	Weight (g)	U (p p m)	Pb (p p m)	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	Blank* (%)	μ†
Chip A	0 88883	0 0528	0 5532	13 780 ±0 020	12 331 ±0 020	34 019 ±0 050	0 5	5 04
Chip B	0 77983	0 0486	0 5238	13 566 ±0 010	12 187 ±0 010	34 712 ±0 030	0 6	4 93
Whole meteorite powder	0 50644	0 0372	0 3521	13 588 ±0 030	12 204 ±0 030	34 120 ±0 080	1 4	5 56

The data are corrected for analytical blanks, and the errors quoted (at the 1σ level) include uncertainties from the blank correction
*Pb blank divided by the total Pb in the sample used
†Measured $^{238}U/^{204}Pb$ ratios

of model Rb–Sr 'ages' as part of their argument that Nakhla originated in a differentiated planetary body not unlike the Earth, and in particular to argue that this parent body suffered gross differentiation between 3 6 and 1 37 Gyr ago We show that the U–Pb systematics of Nakhla are inconsistent with one or more of the assumptions required for the calculation of a model U–Pb 'age', thus indicating that model 'ages' for other radioactive decay schemes may be generally invalid

Table 1 shows U and Pb contents and the atomic ratios $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ determined for each of three bulk samples of Nakhla The techniques used are described elsewhere[5,6], U concentrations are determined to about ±1% and Pb concentrations to about ±1 5% Blank levels were about 0 05 ng U and 2 ng Pb It is clear that single stage model 'ages' are close to 4 01 Gyr (Table 2), indeed the mean model 'age' is 4 01 ± 0 09 Gyr, where the error quoted is twice the standard

Table 2 Radiogenic $^{207}Pb/^{206}Pb$ ratios and single-stage model ages for Nakhla

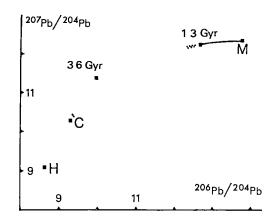| Sample	$^{207}Pb/^{206}Pb_R$	Single-stage 'ages' in Gyr		
		$^{207}Pb/^{206}Pb$	$^{206}Pb/^{238}U$	$^{207}Pb/^{235}U$
Chip A	0 45540	4 103	4 096	4 100
Chip B	0 44447	4 067	4 014	4 049
Whole meteorite powder	0 44616	4 073	3 681	3 938

error of the mean In these calculations the initial Pb isotope ratios are Tatsumoto's revised values for Pb in the troilite (FeS) of the Cañon Diablo iron meteorite[7], and the latest values for the U decay constants are used[8]

Model 'age' calculations are used when the initial isotopic ratio of a daughter element cannot be obtained directly from measurements, for example, in the U–Pb and Rb–Sr systems 'primitive' Pb and Sr isotopic ratios of meteorites have been combined with lunar, terrestrial and meteoritic measured values to obtain the 'age' of the early lunar differentiation[9–11], the 'age' of the Earth[12,13] (U–Pb only) or the 'ages' of certain stony meteorites[7], respectively The various assumptions which must hold for a model 'age' calculation to be valid when the U–Pb system is used, are as follows

(1) The Pb isotope ratios of Cañon Diablo troilite represent the initial ratios of the material being studied

(2) The ratio $^{235}U/^{238}U$ has changed with time by radioactive decay only, and is at present 1 137 88

(3) Changes in Pb isotope ratios were brought about by U decay only

(4) The system has remained closed, that is, the U/Pb ratio has not changed by chemical fractionation

Many meteorites suffered a thermal event about 4 56 Gyr ago[14,15] and Pb isotopic dating is broadly consistent with the assumption that Cañon Diablo troilite has retained the Pb isotopic ratios which generally obtained at that time[16] If Nakhla is composed of matter which evolved in the Solar

System, as did the stuff of other meteorites, then 4 56 Gyr ago, it too, or its parent body, should have had the same Pb isotopic composition as other meteorites Lead of the isotopic composition found in Cañon Diablo troilite could, however, not have evolved in a single stage into modern Nakhla Pb in a time other than about 4 01 Gyr unless the isotopic composition of its U was different from the accepted one (see (2)) and/or it had preferentially lost or gained ^{207}Pb or ^{206}Pb, that is, either or both of assumptions (2) and (3) did not obtain If Nakhla experienced a two-stage history, it could have evolved from the Cañon Diablo composition at 4 56 Gyr, but only provided that the first stage had a U/Pb ratio of zero (usually expressed as zero μ, $^{238}U/^{204}Pb$) and the second stage lasted for about 4 01 Gyr This implies that the Nakhla parent body was free of U from 4 56 to 4 01 Gyr, when U was introduced without the introduction of radiogenic Pb This seems improbable It is possible to invent three-stage (or higher order) histories which allow Nakhla to evolve to its present U and Pb content and Pb isotopic composition from the Cañon Diablo composition over 4 56 Gyr, but we have no evidence of such histories In any case assumption (4) would have been violated Thus, if assumptions (2), (3) and (4) did hold, the initial isotope ratios of Nakhla or its parent body were different from Cañon Diablo troilite Pb (assuming that this composition is linked to the time 4 56 Gyr), that is, assumption (1) did not hold For Nakhla, at least one of assumptions (1)–(4) is false

Fig 1 Nakhla (chip A) Pb growth two interpretations *a*, Single stage, curve HCM, observed μ = 5 04 Growth begins 4 56 Gyr ago from H with hypothetical primitive values, $^{207}Pb/^{204}Pb$ = 9 1 and $^{206}Pb/^{204}Pb$ = 8 6, passes through C, the Cañon Diablo point, 4 1 Gyr ago and continues to M, with present-day measured ratios *b*, Three-stage curves (diagrammatic) C–3 6 Gyr–1 3 Gyr–M (1) From Cañon Diablo values, growth begins 4 56 Gyr ago with μ = 1 81 till 3 6 Gyr ago, when $^{207}Pb/^{204}Pb$ = 11 0 and $^{206}Pb/^{204}Pb$ = 9 8 (2) Growth continues with μ₂ = 5 46, till 1 3 Gyr ago, when $^{207}Pb/^{204}Pb$ = 12 3 and $^{206}Pb/^{204}Pb$ = 12 7 Here the single-stage curve is rejoined (3) From 1 3 Gyr ago, with measured μ₃ = 5 04 till the present We emphasise that we have no evidence for selecting any particular three-stage model, nor do we have evidence for the hypothetical primitive point

The simplest, single-stage explanation is that the Nakhla Pb isotopic ratios grew by U decay over the past 4 56 Gyr from Pb more primitive than that of Cañon Diablo troilite and with the following approximate ratios $^{207}Pb/^{204}Pb = 9\ 1$ and $^{206}Pb/^{204}Pb = 8\ 6$ (Fig 1) The existing U–Pb data are insufficient to prove whether or not this explanation is valid for Nakhla, or to reconcile it with the evidence from the Rb–Sr system[4], it is mentioned here merely as a possibility By chance, Papanastassiou and Wasserburg's interpretation of their Rb–Sr data[4] could be compatible with a three-stage model based on our U–Pb data and Cañon Diablo initial lead isotopic ratios at 4 56 Gyr, U–Pb fractionation could have occurred at about 1 37 and 3 6 Gyr (Fig 1) The second event, which these authors interpreted as representing internal redistribution of Rb and Sr, must, however, necessarily have changed the μ value in the bulk meteorite It seems likely that any major change in the U/Pb ratio would also be accompanied by change in the Rb/Sr ratio so obliterating the record in the Rb–Sr system The choice of 3 6 Gyr as the time for the earlier fractionation must therefore be arbitrary and is not specifically required by the U–Pb data Indeed, we have established a 16 point internal isochron for the Nakhla Rb–Sr system which confirms this interpretation (to be published)

Because the Rb–Sr system has a single parent and single daughter isotope, in theory any value of the ratio $^{87}Sr/^{86}Sr$ could have developed by Rb decay from any less radiogenic initial ratio Thus the applicability of a particular 'initial' $^{87}Sr/^{86}Sr$ ratio of meteorites, such as BABI[17], to determining model 'ages' (which, in the literature, are almost always single-stage 'ages') of other materials can be neither proved nor disproved It seems likely, however, that Rb–Sr model 'ages' might well suffer from uncertainties similar to those shown to exist in the U–Pb systematics of Nakhla, and so it is desirable that meteorite chronologies be based on internal isochrons using both the Rb–Sr and U–Pb systems

The systematics of Nakhla clearly demonstrate that the widely accepted 'primitive' Pb isotope ratios of Cañon Diablo troilite cannot represent initial values for single-stage evolution of the U–Pb systems of this other meteorite The use of Cañon Diablo Pb in determining the 'ages' of lunar rocks (see, for example, ref 11), the 'age' of the Earth[13,18,19] or the 'ages' of stony meteorites[19,20] by single-stage calculations might also be invalid We are merely reiterating a reservation already expressed by numerous earlier workers (see, for example, refs 11, 13, 19 and 20), but we do so with added proof

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Ages of fossil bones from British interglacial sites

THE time gap between the upper limit of radiocarbon dating ($\approx 60{,}000$ yr BP) and the lower limit of dates generally obtainable using the K–Ar method ($\approx 250{,}000$ yr BP) accounts for the scarcity of dates for the last two interglaciations (the Ipswichian and Hoxnian of Britain, the Eemian and Holsteinian of northern Europe) Accordingly, the ages of such important fossils as the Swanscombe and Steinheim skulls can only be guessed at For that reason, the adaptation of a method that may date these interglacial periods is highly desirable We discuss here the application of a uranium-series dating technique pertaining to that span of time

Results were obtained for six samples of fossil bones from British interglacial sites (Table 1) Fossil bones can be dated using the uranium-series method by taking measurements of uranium isotopes and their long-lived daughters, ^{230}Th and ^{231}Pa (refs 1–4) The bone samples described here were cleaned by scraping and by ultrasonic scrubbing The samples were crushed to a fine powder and ignited at 800 °C for about 6 h The abundances of uranium and thorium were determined using mass spectrometry, the $^{234}U/^{238}U$ and $^{230}Th/^{234}U$ activity ratios were determined using α spectrometry[5] The $^{231}Pa/^{235}U$ activity ratios were determined using neutron activation and α spectrometry[6]

Sample localities, analytical data, and calculated ages are given in Tables 1 and 2 The uranium content of the bones varies from 23 to 67 p p m The concentration of common thorium, ^{232}Th, is low in all the samples, therefore, no correction for the initial ^{230}Th component is required The measured $^{230}Th/^{234}U$ ratio yields a ^{230}Th date of $245{,}000 + 35{,}000 - 25{,}000$ yr BP for sample 37 from the Clacton locality The $^{231}Pa/^{235}U$ ratio in this sample is $1\ 00 \pm 0\ 04$, indicating that this fossil apparently remained a closed system with respect to uranium and its daughter elements, ^{230}Th and ^{231}Pa, during at least the last 150,000 yr of its history The calculated ^{230}Th date therefore seems to be reliable

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Table 1 Sample descriptions

Sample 37, Clacton, Essex (OS ref TM 157134) Find no 243 of 1969 Clacton Golf Course Excavations (University of Chicago), cutting xii, layer 4 Sample from gravel below variegated marl, 1 47 m below surface[12,22] Gravel contains typical Clactonian assemblage and Clacton fauna, equals gravel under variegated marl in Oakley's 1934 excavation, but here shelly sand with *Valvata antiqua* separates the gravel and the marl

Sample 38, Barnfield Pit, Swanscombe, Kent (OS ref TQ 598742), collected from lower gravel Lower Barnfield stage of Oakley

Sample 39, Barnfield Pit, Swanscombe "A few cm below skull horizon" Base of upper middle gravel, containing rich fauna and Acheulian assemblage[13] Middle Barnfield stage of Oakley

Sample 63 Barnfield Pit, Swanscombe Find no 82 in 1972 excavations[23] Trench C 3, 1 88 m below datum Middle of Lower Loam, 90 cm below top and about 110 cm above top of lower gravel Lower Barnfield stage

Sample 41, Stutton, Suffolk (OS ref TM 149338) From Brickearth 50–60 cm above beach and immediately above mollusca-bearing levels[24] Strata in ascending order 0 3 m OD pollen of zone III, Ipswichian, 0 6–0 9 cm OD brackish mollusca, 1 0 m OD *Corbicula* maximum, 1 0–2 0 m OD *Corbicula* decline, level of bone sample about 2 5 m OD

Sample 42, Brundon (Jordan's pit), Essex, close to Suffolk border (OS ref TL 860420) Sample marked "21 2 13 pit by railway towards Brundon" Fossil mammals occur in gravel with freshwater mollusca including *Corbicula*, and are underlain by till[25] Mollusca and mammals represent temperate conditions, but include much mammoth Stone artefacts include hand axes and tortoise core

Table 2 Analytical data and calculated dates of fossil bones from British interglacial sites

Sample	Locality	U (p.p.m.)	Th (p.p.,m.)	$\frac{^{234}*U}{^{238}U}$	$\frac{^{230}*Th}{^{234}U}$	$^{230}†Th$ date (yr)	$\frac{^{231}*Pa}{^{235}U}$	^{231}Pa date‡ (yr)	Uranium series date (yr)
37	Clacton	45.5 ± 0.5	<0.03	1.26 ±0.02	0.946 ±0.038	245,000 +35,000 −25,000	1.00 ±0.04	>150,000	245,000 + 35,000 − 25,000
38	Swanscombe	43.7 ± 0.4	0.24 ±0.04	1.48 ±0.03	1.31 ±0.05		1.44 0.06		
39	Swanscombe	67.3 ± 0.7	0.039 ±0.002	1.24 ±0.02	1.01 ±0.04	326,000 + 99,000 − 54,000	1.01 ±0.04	>164,000	>272,000
63	Swanscombe	42.6 ± 0.4	0.18 ±0.01	1.35 ±0.02	1.21 ±0.05				
41	Stutton	22.7 ± 0.2	0.048 ±0.002	1.21 ±0.02	0.808 ±0.032	165,000 ± 14,000	1.14 ±0.06		125,000§ ± 20,000
42	Brundon	58.8 ± 0.6	0.019 ±0.001	1.21 ±0.02	0.919 ±0.037	230,000 ± 30,000	1.17 ±0.06		174,000§ ± 30,000

*Experimentally measured activity ratios.
†Calculated using ^{230}Th half life of 75,200 yr.
‡Calculated using ^{231}Pa half life of 32,500 yr.
§Open-system date after Szabo and Rosholt[5].

Two samples from Swanscombe could not be dated. In sample 38, both the ^{230}Th/^{234}U and ^{231}Pa/^{235}U ratios are anomalously high (1.31 ±0.05 and 1.44 ±0.66, respectively). That is, the amounts of the daughter elements ^{230}Th and ^{231}Pa are in excess with respect to their radioactive parents, ^{234}U and ^{235}U. Accordingly, no ^{230}Th or ^{231}Pa dates can be calculated; and the open system model, which allows for the migration of uranium, also yields no finite date[5]. The ^{230}Th/^{234}U ratio of 1.21 ±0.05 in sample 63 is also too high to yield a ^{230}Th date. Sample 39 from Swanscombe, on the other hand, has a measured ^{230}Th/^{234}U ratio of 1.01 ±0.04 which yields a finite ^{230}Th date of 326,000 +99,000 −54,000 yr BP. The ^{231}Pa/^{235}U ratio of 1.01 ±0.04 indicates that this sample remained a closed system during at least the last 164,000 yr of its history. The large error of the ^{230}Th date arises because the calculated date is near the limit of resolution of the method.

Sample 41 from Stutton and sample 42 from Brundon yield finite ^{230}Th dates but the amount of ^{231}Pa in both cases is excessive with respect to the parent isotope ^{235}U. Using the open system model the dates of sample 41 from Stutton and sample 42 from Brundon work out at 125,000±20,000 and 174,000±30,000 yr BP, respectively.

The Clacton deposits, including artefacts, mammalia and mollusca, have been placed by most workers in the Hoxnian Interglaciation[7-9] although Collins[10] and Kerney[11] have argued for an early–middle Hoxnian age (zone II). Singer et al.[12] have, however, suggested that the gravels belong in the Anglian Glaciation. The Swanscombe skull level is also placed by most workers in the Hoxnian[7,9,13], though possibly very close to the end (zone IV or late III)[10,11].

The Stutton deposits, only some 8 km from the type site of the Ipswichian[9], are usually placed in the Ipswichian[7]. If, however, a temperate interval existed during the glaciation between the Hoxnian and Ipswichian, the Stutton deposits could possibly be correlated with this interval. Similarly, the Brundon faunal level, only tenuously connected with the Ipswichian in the absence of palaeobotanical data, though clearly post-Hoxnian, may correlate with a possible temperate interval between the Hoxnian and Ipswichian.

Among these samples only sample 37 from Clacton gives a satisfactory closed system finite date, of 245,000 yr BP. This date agrees with a 'medium chronology' for the Pleistocene interglaciation. The date is too old for the short chronology of Emiliani[14], and too young for the long chronology of Ericson et al.[15]. The Swanscombe date may be regarded as a minimum, at least 272,000 yr BP. Most chronologies would require Swanscombe to be later than Clacton; the circumstance can be reconciled for the samples reported here by adopting the older limit for Clacton, that is about 280,000 yr BP.

The Brundon date, 174,000±30,000 yr BP (open system), is older than most recent estimates for the last interglaciation, even the 120,000 yr age favoured by Shackleton[16]; it would correlate well with a Wolstonian temperate period (inter-Hoxnian–Ipswichian), or with the Ipswichian, if a longer chronology is assumed. The Stutton date, 125,000±20,000 yr BP (open system), is very close to Shackleton's[16] estimate for the Ipswichian. Within the limits of statistical error, the Stutton date would also agree with a date of about 100,000 yr BP for the Ipswichian. Alternatively, this date, like that from Brundon, would correlate with a hypothetical inter-Wolstonian temperate period at about 145,000 yr BP; this leaves the Ipswichian to be mainly younger than 100,000 yr BP, as has been widely suggested.

The Clacton date may be compared with two K–Ar dates that bear somewhat indirectly on the age of the Holstein (Mindel–Riss) Interglaciation in Europe: the KA 409 date[17] from Monte Cavo, 20 km south-east of Rome, which dates leucite from a post-Flaminian (Mindel/Elster) eruption at 277,000 yr BP; and a Leilenkopf III date of 220,000±40,000 yr BP on lava from an eruption some 36 km SSE of Bonn in the 'mittlere Mittel' terrace of the Rhine[18], which is roughly contemporary[19] with the beginning of the Holstein Interglaciation. The Clacton, Monte Cavo, and Leilenkopf dates are all consistent with a date of about 250,000 yr BP for the early part of the Holstein Interglaciation, if allowance is made for the full range of statistical error.

The four dates reported here support a medium chronology (see refs 10, 20 and 21) for the Pleistocene interglaciations. The dates do not support the short[14,18] or long[15] chronologies. A date of about 250,000 yr BP would seem to be the best estimate at present for the time of the Holstein Interglaciation.

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Subglacial silica deposits

SUPERFICIAL carbonate deposits are common on bedrock surfaces recently exposed by retreating temperate glaciers[1-5]. Their morphology indicates that they formed subglacially in intimate contact with sliding ice. Besides suggesting that chemical transport is an active subglacial process, these relatively widespread deposits reflect high solute concentrations in subglacial waters. This is a fact of considerable significance because solutes at the glacier–bed interface can impede significantly the sliding of temperate glaciers[5]. The dynamics of temperate glaciers and especially the more intriguing aspects of their behaviour, such as surging[6], are thought to depend critically on the basal sliding process[7]. Thus, chemical exchange at the base of a glacier may affect significantly its entire motion. Moreover, because glacial erosion and deposition are largely dependent on glacial sliding, they too are affected by the presence of solutes at the bed. To date, reported subglacial deposits have all been carbonates. On the basis of previously unrecognised subglacial deposits rich in silica, I suggest that subglacial deposits are not exclusively carbonates, but that

silicates are of wider significance, than is believed at present.

Figure 1 shows a sample of hypersthene–augite andesite, from the vicinity of Paradise Glacier in Mount Rainier National Park, Washington. This rock has a thoroughly striated and polished surface which merges in the down-glacier direction into a slightly irregular surface, coated with a thin light grey layer of mineral material. The surface of the coating is characterised by a pronounced lineation parallel to the local glacial striations, suggesting that it formed in intimate contact with actively sliding ice in much the same way as carbonate deposits[5].

This millimetre-thick deposit consists of two contiguous, distinct types of material that differ markedly from the underlying bedrock. The more abundant type consists principally of angular, micrometre-sized rock and mineral fragments, presumably glacial flour, embedded in an amorphous matrix. The other type of material is finely laminated and contains only a few small clastic fragments. It ranges in colour from light to
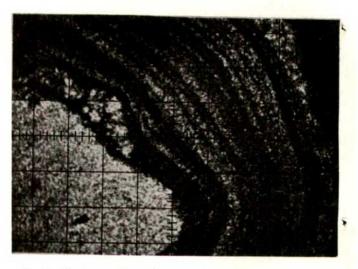

Fig. 2 Electron-probe, sodium-sensitive micrograph of feldspar phenocryst in andesite bedrock partially coated with finely laminated, subglacially precipitated, silica rich coating. The lighter areas are relatively rich in sodium. Grid spacing, 25 μm.

dark brown in transmitted light and is characteristically optically isotropic, although in places it may be slightly birefringent. Electron microprobe analyses (EMA) indicate that the deposit is highly siliceous, in places consisting of as much as 95 % SiO_2.

Figure 2 is an oscilloscope presentation of the characteristic X-ray radiation of sodium from an electron microprobe scan of the finely laminated, optically isotropic material partially coating a feldspar grain. The lamination is clearly compositional in nature with alternating layers relatively richer and poorer in sodium. A silicon micrograph of this same area revealed little structure in as much as the entire deposit is rich in SiO_2.

An X-ray powder diffraction pattern of the silica-rich coating showed sharp plagioclase peaks, presumably representing detrital feldspar grains in the deposit. There were no diffraction peaks of quartz or other silica polymorphs in the powder patterns. Transmission electron microscopic (TEM) examination of the deposit revealed a few submicrometre sized and larger, angular, mineral fragments embedded in a homogeneous matrix. No crystallographic subdomains could be detected in the matric at magnifications of up to ×83,000. Electron-diffraction patterns of the silica deposit invariably consisted of several families of spots, interpreted as a result of the presence of detrital grains of feldspar and ferromagnesian minerals. If the silica-rich matrix consists of crystalline subdomains, they are too small to cause significant diffraction of the electrons. Thus, the SiO_2 in the subglacially formed deposit is either

Fig. 1 Glacially polished andesite surface on the right grades to the left into an irregular surface covered with a thin furrowed coating that formed in intimate contact with sliding temperate ice. Former direction of ice flow is indicated by the arrow, which is 2 cm long.

amorphous, or else the size of the ordered domains does not exceed several hundred angstroms

Insight into the mode of formation of the subglacial silica-rich deposits can be obtained by analogy with previous studies of the subglacial precipitation of $CaCO_3$, which is intimately related to the basal sliding of temperate glaciers[1-5] These glaciers, at their melting points throughout, flow by a combination of two processes internal deformation and basal sliding Sliding ice accommodates itself to irregularities in the bedrock by plastic deformation and by regelation In the latter process, the basal ice melts in zones of higher pressure and reduced temperature, and the resulting water flows between the ice and bedrock toward zones of lower pressure and higher temperature, where it refreezes because of the pressure reduction The heat necessary for the fusion is provided by the latent heat of freezing, which is conducted through ice and rock from the lee to the stoss sides of bed obstacles[8,9] The meltwater continuously produced by regelation sliding contains impurities originally present in the basal ice in addition to these resulting from the dissolution of the bedrock As this water refreezes in areas of locally reduced pressure, generally along lee surfaces, solutes are continuously rejected into the liquid phase by the growing ice The continuous rejection of dissolved silica, which is probably similar to the rejection of solutes in freezing NaCl (ref 10), KOH (ref 11), HCl (ref 12), and $CaCO_3$ (ref 5) solutions, and which must occur in the sliding process, will therefore cause progressive enrichment and eventual precipitation of silica from regelation waters in the lee of bed obstacles at the bases of actively sliding temperate glaciers Solutes are concentrated subglacially in much the same way as in the zone melting process used for refining metals and semiconductors, in this process impurities are removed preferentially by the passage of a molten zone in which impurities tend to accumulate

Concentrations of dissolved silica as high as 8 p p m are present in the water emerging from under two Mount Rainier glaciers[13] Regelation melt water, flowing in intimate contact with the bedrock, should also contain high concentrations of dissolved silica The subglacial, silica-rich deposits indicate that a portion of this mobilised silica is deposited, as an essentially amorphous precipitate trapping much glacial flour, at a temperature very close to 0 °C, a pressure not exceeding a few bars, and a pH close to 7 (ref 13) The deposit I have mentioned (Fig 1) formed recently, perhaps during the last major glacial advance, around 1850 AD (ref 14)

Solutes in regelation waters tend to inhibit glacial sliding because they accumulate at the lee of bed obstacles where freezing is localised They reduce selectively the temperature there, thereby reducing the temperature gradient and thus the heat flow across bed obstacles that is essential for regelation sliding Concentrations of silica exceeding the saturation value of 80 p p m (ref 15) can be expected where amorphous silica is precipitating subglacially Together with other dissolved species generally present[13], a difference in the concentration of solute of this magnitude between stoss and lee surfaces can be expected to inhibit glacial sliding significantly, especially if the glacier bed is characterised by uneven elements which have mean wavelengths that do not exceed 0 5 m (B H , unpublished)

Subglacially formed carbonate deposits are relatively widespread, having been recognised on calcareous rocks in Scandinavia[3], the Alps[4], and the Rocky Mountains[1,5] This is the first report of subglacial silica-rich deposits They are probably not restricted to the Mount Rainier locality, but rather, are likely to be widespread perhaps as thinner coatings and inconspicuous crack fillings, in glaciated areas underlain by silica-rich rocks Considerable amounts of solutes are, therefore, likely to be present to the lee of bed obstacles at the base of practically all temperate glaciers, because most rocks are rich in carbonates or silica, Thus, the effect on glacial sliding is of general importance, because it will have a definite influence on the behaviour of practically all temperate glacial ice masses

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Observations of a nonlinear interaction involving three electromagnetic waves in a laboratory magnetoplasma

POLARISATION requirements and wave number matching dictate that a nonlinear interaction involving three electromagnetic waves that propagate in a magnetoactive plasma must necessarily be two-dimensional if one of the waves propagates parallel to the external magnetic field B_0 We report here what we believe to be the first observation of a three wave interaction of this kind Our experiment involved the production of a whistler mode wave (k_3, ω_3) (referred to as WMW) through the interaction of two beams of high frequency electromagnetic waves (k_1, ω_1) and (k_2, ω_2), propagating somewhat above the local plasma frequency ω_p The resonance conditions for the interaction are

$$k_1 = k_2 + k_3 \text{ and } \omega_1 = \omega_2 + \omega_3 \qquad (1)$$

If $\omega_{1,2} > \omega_p$ and ω_3 is sufficiently small compared with the electron gyrofrequency ω_e $(\omega_e < \omega_p)$, the high frequency beams must be directed[1] almost at right angles to B_0 (Fig 1 inset) The strength of the coupling of the beams is determined by two factors first, the magnitude of the matrix element for the interaction, and second, the time in which a stationary state is established as a result of the convection of the WMW packet across the finite interaction region of width, l An expression[2,3] for the steady-state amplitude, E_3 of the electric field of the WMW, can be obtained in terms of the amplitudes E_1 and E_2 of the beams

$$E_3 = \chi E_1 E_2 \qquad (2)$$

where

$$\chi = \frac{1}{2} \frac{el}{mc^2} \frac{\omega_3^2}{\omega_1 \omega_2} \frac{\omega_e \mu^2}{(\mu^2 \omega_e^2 + 4\omega_1^2)^{1/2}}$$

where e and m are the charge and mass of the electron, respectively, c the speed of light, μ, the refractive index for the WMW, and other symbols are as defined previously

The laboratory plasma is produced by transverse r f excitation of Argon gas in a 1-m long, 15-cm diameter, glass tube immersed in an axial magnetic field variable from 20 to 200 gauss, and uniform to within 2% over 60 cm length This
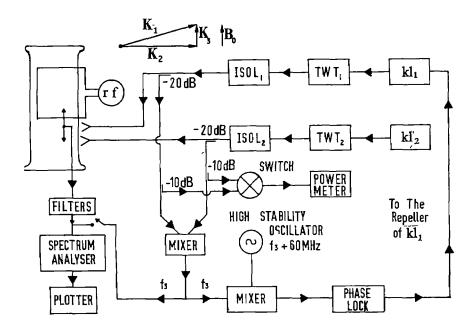
Fig. 1 Schematic diagram of the apparatus used in our experiment For the true disposition and orientation of the horn antennae see text Vector diagram at the top shows wave vector configuration for the interaction $(\mu_{1,2}\sim1, \mu_3\gg1)$

method of plasma production[4,5] creates a plasma with electron temperature $T_e\sim3$ eV and density $n_e\sim10^{12}$ cm^{-3} Langmuir and wave field probes can be inserted radially into the plasma and a motor driven probe is used for the detection of the wave field along the tube axis

Two microwave beams at frequencies f_1 and $f_2\sim11$ GHz are derived from two Klystron oscillator and TWT amplifier systems, and fed into the plasma through two horn antennae, which are situated side by side around the circumference of the tube The beam from one horn (f_1) is oriented perpendicularly to the axial magnetic field with the electric vector E_1 at right angles to B_0, and the other (f_2) is oriented at 5-10° off the perpendicular, with E_2 parallel to B_0 The power in each beam is controllable up to 15 W
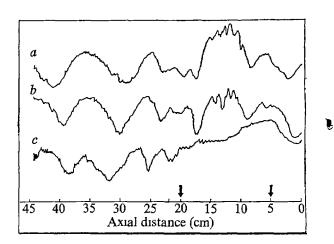
The frequency difference $f_3 = f_1-f_2$ is stabilised using a double down converting system, locked to a 60 MHz standard which controls the repeller voltage of one of the Klystron oscillators, and can be varied at will in the range 0-400 MHz As the deviation rate of the residual f m in the Klystrons is relatively slow, the response rate of the phase locked system is more than adequate to achieve overall frequency stabilisation to within some tens of Hz Narrow band detection and frequency stabilisation are essential in the somewhat noisy plasma, and considerable care has been taken in eliminating pick-up

Signals at f_3 are detected using the axial loop probe and a spectrum analyser (see Fig 1) The combination of the frequency locking system and the stabilisation on the spectrum analyser results in an overall bandwidth at f_3 of ~20 Hz, with a sensitivity of -120 dBm, which corresponds to a minimum detectable oscillating magnetic field of 10^{-8} gauss for the frequencies we are interested in Figure 2 shows the results of interferometric measurements of wave amplitude as a function of axial position of the probe, for $f_3 = 43$, 51 and 67 MHz, while the plasma conditions were kept constant The two arrows on the figure define the length $(l\approx15$ cm) of the interaction region, that is, the length of the plasma volume illuminated by the two microwave beams At a short distance from this region the signal exhibits a clear phase variation with distance, from which the wavelength can be deduced After allowing for small changes in wavelength $(\sim10\%)$ related to a weak beach field in the last 10 cm of the detection region, we have fitted (Fig 3) the experimental points to the low frequency WMW dispersion relationship appropriate to the geometry of this experiment[6,7] The average

plasma density $n_e = 1\,2\times10^{12}$ cm^{-3}, deduced from this fit, is in good agreement (to better than 20%) with the density deduced from Langmuir probe measurements

The same figure also shows the dispersion relation for quasistatic waves[8] which can exist in this experiment under the same plasma conditions The identification of the waves at f_3 with a WMW is unambiguous The existence of long wavelength, quasistatic waves in our apparatus was indeed established during preliminary investigations, by observing (in separate measurements without a reference signal in the spectrum analyser) the beating between short wavelength WMWs $(\lambda_3\sim15$ cm) and quasistatic waves $(\lambda_{q\ s}\sim200$ cm) We have also verified that the WMW field is proportional to the product of the input fields, and that for a fixed orientation of the high frequency beams, the amplitude of the WMW field varied with varying plasma parameters as predicted by the theory,

Fig. 2 Interferometer traces of WMW fields, detected by the loop probe, at three different frequencies, as a function of axial distance a, $f_3 = 43$ MHz, b, $f_3 = 51$ MHz, c, $f_3 = 67$ MHz Arrows on the distance scale mark the approximate extent of the region over which the microwave beams interact The maximum amplitude corresponds to an electric field $E_e\sim$ 3×10^{-4} V m^{-1}

that is, it exhibits a broad resonance centred on the resonant condition of equation (1)

Care was taken to match the microwave beams at the glass-plasma interface, and microwave absorbers were packed around external sources of reflection Nevertheless, some internal reflection did occur, and the possibility that regions of enhanced microwave field could give rise to other non-linearities (for example, on the sheaths, probes, connections, and so on) and produce spurious signals at f_3 with the same spatial structure was investigated These fields were examined by scattering measurements, and detection on the loop probe, and shown to be at least 30 dB down on the fields in the interaction region and to have a spatial structure along the axis totally unrelated to those obtained by phase measurements

The magnitude of the coupling coefficient for the present experiment can be obtained by substituting the measured values of the amplitude of the high frequency beams ($E_1 \approx E_2 \approx 300 \text{ V m}^{-1}$) and the amplitude of the emerging WMW ($E_3 \sim 3 \times 10^{-4} \text{ V m}^{-1}$) into equation (2) This gives

$$\chi_{\text{exp}} = 3 \times 10^{-9} \text{ m V}^{-1}.$$

On the other hand, the value of χ predicted theoretically from equation (3), using the experimental values for ω_1, ω_2, ω_p and ω_e gives

$$\chi_{\text{th}} \sim 1 \ 3 \times 10^{-9} \text{ m V}^{-1}$$

The good agreement between these two figures confirms the validity of the weak coupling model for this interaction
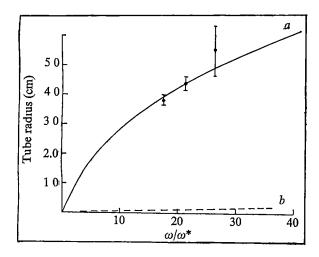

Fig. 3 Experimental data fitted to the theoretical dispersion curve (a) of low frequency whistlers (helicon waves[6]) $\omega^* = B_0/4\pi nea^2$ The inferred value of the plasma frequency, n ($\sim 1 \ 2 \times 10^{12} \text{ cm}^{-3}$) is in good agreement with independent Langmuir probe measurements[7] b, Dispersion curve for quasistatic waves

The original motivation for this experiment was to verify the theoretical predictions concerning the feasibility of an active field experiment[1] to excite WMWs in the Earth's ionosphere by means of two ground based high frequency transmitters of equal beam strengths ($E_{\text{ion}} = E_1 = E_2 = 1 \text{ V m}^{-1}$) and illuminating a region such that $l_{\text{ion}} = 2 \times 10^4 \text{ m}$

It is interesting to extrapolate the laboratory measurements of χ to the ionospheric situation described here This can be achieved by noting that the coupling coefficient for equal beam strengths scales as El (where $E = E_1 = E_2$), if the ratios

ω_3/ω_p, $\omega_{1,2}/\omega_p$ and ω_p/ω_e are kept constant We then find that

$$\chi_{\text{ion}} = \chi_{\text{lab}} \left[(El)_{\text{ion}}/(El)_{\text{lab}} \right]$$

$$= 1 \ 3 \times 10^{-6} \text{ m V}^{-1}$$

which is in good agreement with values calculated previously[1]

Finally, the positive results of this laboratory experiment seem to substantiate the suggestion that the very low frequency bands observed[9] from the satellite Ariel 3 (and subsequently[10], from Ariel 4) over the magnetically conjugate area of the eastern USA may be caused by the interaction in the - Earth's ionosphere of powerful, medium wavelength signals (the f_1 and f_2 phases of our experiment) from commercial transmitters concentrated in that region, which excites WMWs (the f_3 of our experiment) which subsequently undergo natural amplification by a mechanism similar to that observed by Helliwell[11]

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Vacancy migration and void formation during annealing of electron irradiated molybdenum

WE have used a combination of three techniques—resistivity, positron annihilation and transmission electron microscopy (TEM)—to examine the recovery on annealing of point defect damage introduced into high purity molybdenum by 10 MeV electron irradiation The work produced two points of particular interest, both arising directly from the sensitivity of the positron annihilation technique to vacancy defects

The first point concerns the well known stage III recovery occurring in molybdenum at between 150 and 300 °C following either cold work[1,2], neutron irradiation[3,4], or electron irradiation[5,6] That this recovery stage is caused by the annihilation of vacancies with interstitials is little disputed but in molybdenum, as in the face centred cubic (f c c) noble metals, there has been controversy as to the nature of the migrating species[4] We therefore irradiated molybdenum specimens at 50 °C to a dose of 2 4 × 10^{18} electrons cm^{-2} and then combined liquid helium resistivity measurements with room temperature positron annihilation measurements to follow the changes occurring
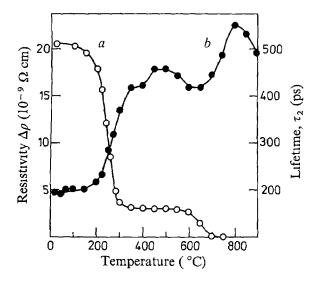
Fig. 1 The recovery of induced resistivity (*a*) and the behaviour of the long positron lifetime, τ_2 (*b*), during the isochronal annealing of electron irradiated molybdenum Heating rate 1 °C min^{-1}

during stepwise isochronal annealing (Fig 1) The resistivity measurements showed that $\sim 80\%$ of the induced resistivity annealed out in stage III centred at ~ 250 °C indicating, as expected, that the majority of radiation induced defects annihilate in this stage

Meanwhile, in exactly the same temperature range the long positron lifetime increased from 195 ps to 400 ps It is well established that positrons are sensitive to the presence of vacancies and vacancy clusters, the parameters of the clusters, such as shape and size, particularly influence the magnitude of $\tau_2^{7,8}$ On this basis, therefore, the significant rise in τ_2 can only be interpreted in terms of the formation of such clusters Since it is difficult to see how interstitial migration can lead to this formation of vacancy clusters we believe that the stage III recovery in molybdenum must arise from the thermal migration of radiation induced vacancies Models based on the migration of metastable interstitials or the detrapping of interstitials are, therefore, untenable, from the combination of resistivity and positron annihilation data it seems clear that during the vacancy migration a large number of the vacancies are annihilated at immobile interstitials (trapped at impurities during the irradiation^{6,9}) and that also, not unreasonably, a significant fraction of vacancies interact with each other to form clusters

The second point of interest concerns the nature of the clusters, particularly as the 400 ps value for τ_2 suggested strongly that they could be three-dimensional To obtain further experimental information we therefore continued annealing to higher temperatures Figure 1 shows that the two significant stages are the well defined annealing of the remaining radiation induced resistivity at 650 °C, and the 800 °C peak in τ_2 where the long positron lifetime reaches a value of 550 ps before the lifetime parameters return to their pre-irradiation values In the light of results from a previous analogous positron annihilation study on neutron irradiated molybdenum^8 the interpretation of these two stages is comparatively straightforward In the neutron work it was suggested^8, and subsequently confirmed by TEM^{10}, that a large rise in τ_2 in the range 750–900 °C was caused by the thermal coarsening of voids, whereas an earlier shoulder on this peak at 650–700 °C reflected the growth of voids at the expense of thermally shrinking vacancy loops

Thus, it seems that during the stage III vacancy migration the vacancies escaping annihilation at interstitials nucleate clusters in two forms, one planar and one three-dimensional, which then grow to vacancy loops and voids, respectively On annealing, the sensitivity of resistivity measurements to lattice

strain effects allows the thermal shrinkage of the small vacancy loops to be detected, leaving the void behaviour to be followed by the positron lifetime parameters These conclusions are fully supported by investigations using the angular correlation technique (M E *et al*, unpublished)

For further confirmation we cut disc-shaped specimens, suitable for electron microscopic study from the resistivity foils, these were annealed to 900 °C before transmission microscopy examination at 100 keV Small voids with diameters of 20–30 Å were found within the foils, the void concentration was about 10^{13} voids cm^{-3} Of the estimated number of vacancies (70 p p m) surviving the irradiation, certainly not more than 1 % survived the annealing to form voids

It is worth emphasising the importance of these positron annihilation results because they reinforce the usefulness of such measurements in point defect studies Furthermore, it seems possible that outstanding problems concerning other metals— for example, the vacancy–interstitial problem in stage III of the noble metals, or the intrinsic–extrinsic defect argument for stage III in the group V refractory metals—might be profitably examined by adding positron annihilation measurements to the normal range of techniques used in such studies

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Fossil hominid body weight and brain size

Reliable estimates of body weights in early hominids are essential to the study of their brains and body proportions[1] Obviously the evolution of the size of the human brain must be studied in terms of the size of the body Although the endocranial volume is known for many individual fossil hominids, body weight is poorly known The present study is an attempt to estimate body weight of the South African Plio-Pleistocene hominids as accurately as possible from the available fossil evidence East African early hominid postcrania are excluded because of the difficulty in taxonomic assessment, especially within the genus *Australopithecus*

Most previous estimates of body weight for these fossils have been based on visual assessment or extrapolations from stature estimates[1-5] This study attempts to predict body weight directly from skeletal size Of the fossils preserved in South Africa, the vertebrae present one of the best opportunities to do this since adult vertebrae of the same kind are known from both *A africanus* (Sts 14 from Sterkfontein)[5] and *A robustus* (SK 3981 a and b from Swartkrans)[5,6] Also, vertebral size is highly correlated with body weight in humans In my human sample, a measure of cross-sectional area of the last lumbar vertebra has a correlation of 0 72 with body weight A measure of the volume of the last thoracic vertebral centrum has a correlation of 0 70
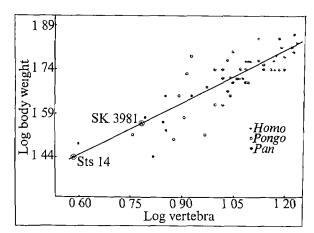
Fig. 1 Regression line and dispersion based on eight measurements of human vertebrae predicting body weight Two male and five female *Pan* and eight female *Pongo* specimens are plotted for comparison purposes only

The Terry collection at the Smithsonian Institution, Washington, provided the comparative human sample This is one of the most extensive skeletal collections, with body weights at death, somatotype photographs, cause of death, sex, age, and other statistics Forty-three specimens were used with about equal numbers from each sex Excessively emaciated or obese individuals are eliminated as well as those over the age of 50 yr For comparison several specimens of orangutan (*Pongo*) and chimpanzee (*Pan*) with known body weights are measured

The sagittal and transverse diameters of the last thoracic and last lumbar centra are taken on the cranial surface and at the middle (corresponding to Martin measurements numbers 4, 6, 7 and 9)[8] The average of the four sagittal diameters is multiplied by the average of the four transverse diameters forming an approximation of the cross-sectional area of the centrum The regression formula

$$\log (\text{weight}) = 0\ 603\log (\text{vertebra}) + 1\ 085$$

has a correlation of 0 69 (Fig 1)

Using this formula the body weight of Sts 14 is estimated to be 27 6±10 5 kg (60 8±23 0 lb) and that of SK 3981, 36 1±10 7 kg (79 3±23 5 lb)

These weights are probably close to the minimum for their taxa The Sts 14 specimen (*A africanus*) is one of the smallest known from Sterkfontein although its estimated body weight is similar to estimates made by other investigators using different methods[1-5] The estimate of body weight for the Swartkrans hominid (*A robustus*) is also probably close to the minimum for that taxon but close to the predictions made by other authors[1-3] It is much smaller than the 68 1–90 8 kg (150–200 lb) estimate derived by Robinson[5] The latter estimates seem excessively heavy since even using the great apes as models, the fossil remains are not consistent with such large weights Figure 1 shows that *Pan* and *Pongo* vertebrae are close to the human regression line Even some of the largest hominid postcranial remains from Swartkrans do not correspond to Robinson's minimum estimate For example, of 14 *Pan* femora measured, the size of the SK 82 femur corresponds to an individual weighing 41 6 kg (91 5 lb) SK 97 is only slightly larger If Robinson's estimates are correct, then the ratio of minimum posterior dentition surface area to body weight in *A robustus* would only be about half that found in *A africanus* which is just the reverse of what is expected[4,9] The other postcranial fossils of the South African *A robustus* indicate a body size smaller than 150 lb (ref 3)

If these body weight estimates are representative and reasonable minima, then the brain to body weight ratios can be calculated with more security than was possible before There are several methods of comparing brain size and body weight Using Holloway's[10] estimates of endocranial volumes (442 cm[3]

for *A africanus* and 530 cm[3] for *A robustus*), the encephalisation quotient[11] is 4 0 for *A africanus* and 4 0 for *A robustus* (compared with *Homo* = 7 1 and *Pan* = 2 5) The index of progression[12-14] is 16 4 and 16 6 in the two fossil forms (*Homo* = 28 8 and *Pan* = 10 4) The constant of cephalisation[12,15] yields values of 42 0 and 47 4 (*Homo* = 103 5 and *Pan* = 35 5) Finally the extra neurone index[16] gives 3 9 billion and 4 4 billion (*Homo* = 8 5 and *Pan* = 3 6)

Figure 2 presents these indices of encephalisation as percentage deviations from *H sapiens* Although there is considerable debate about which of these indices allows the most meaningful comparisons[11-18], a general pattern emerges the two forms of South African early hominids are similar to one another and intermediate between *H sapiens* and *Pan* although much closer to the latter These findings do not support the view expressed by Holloway[19] that the australopithecine brain bears the same relationship to body weight as it does in modern humans The relative size of the brain in these early hominids is very small These fossil forms can be characterised as relatively small-brained but bipedal hominids, a grade in human evolution predicted in general by Darwin, and specifically by Haeckel, in which upright posture precedes brain expansion[5,7,20,21]
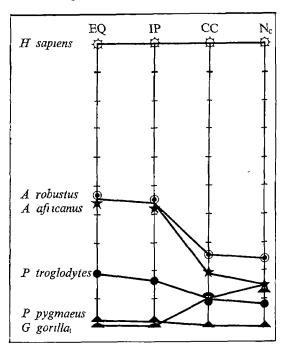

Fig 2 Encephalisation indices expressed as percentage difference from *H sapiens* Comparative data derived from various sources[11-16] EQ, encephalisation quotient[11], IP, index of progression[12-14], CC, constant of cephalisation[12,15], N_c, extra neurones[16]

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How the cerebellum could memorise movements

THE neurones of the cerebellum are arranged in an extremely regular fashion and their synaptic connections are known in detail[1], this has led to considerable speculation about the functioning of these neuronal circuits In particular, following a proposal that the cerebellum is involved in the learning of movements[2], there have been a number of theories about how the cerebellar circuitry could be used in this way[3-5] These theories are all based on the postulate that the signals relating to motor output which are to be stored in the cerebellum have been computed in the cerebral cortex, and transmitted from there to the cerebellum for storage They assume that the initial learning process, during which the animal learns to produce motor outputs with favourable consequences for itself, takes place somewhere in the cerebral cortex and not in the cerebellum only after the cerebral cortex has learned how to generate these motor outputs is the information for their production stored in the cerebellum Recent results suggest, however, that this postulate is incorrect, and that the cerebellum is directly involved in the learning of motor actions which have satisfactory consequences for the animal To account for these results I describe a new way by which the cerebellum could be used for storing information relating to movements

A previous theory[5], which followed an earlier one about learning in single Purkyne cells[3], showed how groups of Purkyne cells arranged as in Fig 1 (called a unit) could be used for this information storage The learning process was postulated to occur by changes in the strengths of the parallel fibre synapses on the Purkyne cells in a unit when the climbing fibres fired It was demonstrated that a unit could learn to respond with a certain output (that is with a particular frequency of firing in the nuclear cell) to a certain parallel fibre input (that is a specific pattern of frequencies of firing in the parallel fibres) This output would then cause movement in a muscle and sensory feedback would create a new pattern of firing in the parallel fibres If the unit had learned to respond to this new input then a new output would be produced and the movement would progress in this manner

This method of memorising movements assumes, however, that the animal already knows how to perform the movements, and can send the appropriate signals required for a movement from the cerebral cortex, by way of the climbing fibres, to be stored in the cerebellum An alternative scheme is suggested by work on the nucleus locus coeruleus Thus it is known that axons from the noradrenaline-containing cells of the locus coeruleus contact Purkyne cells in the cerebellum[6] (as shown in Fig 1), and that stimulation of the locus coeruleus cells inhibits the firing of Purkyne cells[7] Furthermore, the locus

coeruleus is connected with the reward, or positive reinforcement, system of the brain in that it will support intracranial self-stimulation behaviour[8]

Therefore the locus coeruleus could be used in the consolidation of the motor signals stored in the cerebellum This would be done in the following manner When a movement was carried out the changes in synaptic strengths would be the same as those described above, but they would not be made permanent unless a signal was sent by means of the locus coeruleus axons to the Purkyne cells in a unit indicating that the outcome of the movement was satisfactory and that the movement was suitable for storage This signal is presumably caused by increased rates of firing of locus coeruleus cells which then release larger quantities of noradrenaline at their synapses on the Purkyne cells The release of noradrenaline has been shown to lead to the phosphorylation of membrane protein in guinea pig cerebral cortex[9], and the requisite changes in strengths of parallel fibre synapses could possibly be mediated in this fashion in Purkyne cells Although the immediate effect of noradrenaline is to inhibit the firing of Purkyne cells[7], a mechanism like this could lead to a long term increase or decrease in synaptic strengths Crow[10] has proposed that the noradrenaline system could be used for transforming short term synaptic changes into long term changes Because a motor action would be made up by a sequence of outputs from the units of Purkyne cells, there would probably be a signal requiring the synaptic changes corresponding to a number of these outputs to be made permanent simultaneously As the synaptic changes at a parallel fibre synapse required for the storage of successive outputs are additive[5], there is no difficulty in constructing biochemical models for this type of memory storage

Figure 2 shows the different stages involved in the learning process For long term memory, signals are required at a synapse from three separate sources The initial short term
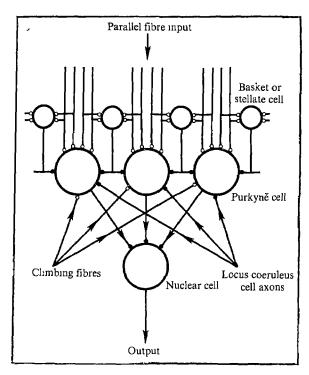
Fig 1 The diagram shows the arrangement of the cells in one of the units involved in memorising movements Each unit contains a large number of Purkyne cells which project to the same nuclear cell, or group of nuclear cells[5] Every Purkyne cell receives excitatory inputs from as many as 200,000 parallel fibres, and inhibitory inputs from basket and stellate cells[1] The Purkyne cells are also contacted by climbing fibres and fibres from locus coeruleus cells The unit can learn to respond to different parallel fibre inputs with specific frequencies of firing in the nuclear cell, through appropriate changes in the strengths of the parallel fibre synapses

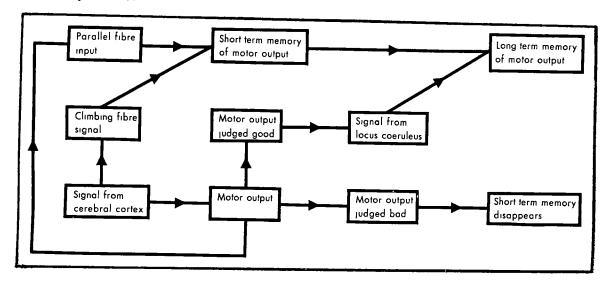**Fig 2** The proposed scheme for the storage in the cerebellum of information relating to movements is shown Initially a movement is carried out through signals from the cerebral cortex These signals cause climbing fibre signals in the memorising units of the cerebellum projecting to the muscles involved in the movement The simultaneous firing of the parallel and climbing fibres produce changes in the parallel fibre synapses on the Purkyne cells giving a short term memory of the movement Only if the movement is judged to be satisfactory will a signal be sent from the locus coeruleus to convert the memory into a long term form

changes in strength of synapses are produced in the cerebellum by the conjunction of signals from two of these sources transmitted by the parallel and climbing fibres The synaptic changes take place at the parallel fibre synapse and it is the parallel fibre input to the cell which is used for the memory recall process The climbing fibre input has a different role it specifies the times at which motor outputs are to be memorised by a unit and later recalled during the learned movement It was originally postulated[5] that the climbing fibre could transmit signals of differing magnitude so that the unit could learn to fire with different output frequencies (this accounted for the observed changes in the frequency of firing of cerebellar nuclear cells during the performance of learned movements[11]) Recent observations of climbing fibre signals during the learning of movements[12] do not, however, support this postulate With the present memorising scheme it is only necessary for a climbing fibre to send signals of one magnitude and cause only one output frequency of firing to be learned by the unit (the memory capacity of a unit is very similar in this situation) If this frequency were not sufficient for the muscular action at a certain point in the movement then a second signal could be sent the next time the movement was practised, and this could be repeated until the movement was judged completely satisfactory The changes in strength at the parallel fibre synapses are not made permanent and converted into a long term memory until a signal is received from the locus coeruleus If the motor output is unsatisfactory and no signal is received then the short term changes in the synaptic strength will decay to zero and there will be no memory of this motor output Presumably the spontaneous firing of climbing fibres about 1–2 times per second[1] causes short term changes of this type which are not consolidated into long term changes

Evidence for this proposed role of the locus coeruleus in the learning of movements comes from a study[13] on the effect of locus coeruleus lesions in rats These lesions abolished the ability to learn to run with increased speed for a food reward, while causing no apparent motor deficit Similarly it has been shown[14] that depletion of brain catecholamines produces a severe impairment in the learning of a complex motor sequence, though once the task is learned there is no retention defect

Various parts of the brain besides the cerebellum are involved in the production of movements, and therefore these studies show that the locus coeruleus may be involved in learning elsewhere in the nervous system This is also indicated by the

widespread distribution of terminals from locus coeruleus cells to regions other than the cerebellum[15]

In conclusion, the cerebellum seems to be a favourable system for studying the neuronal circuitry associated with short term and long term memory The failure to demonstrate learning in the cerebellum at the cellular level[16] could have resulted because no account was taken of the locus coeruleus in these experiments

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Acceptance of novel flavours is increased after early experience of diverse tastes

WE believe that the more an animal experiences diversity of flavours at one time the more it will accept novel flavours later Thus, rats given several distinctive flavours early in life would be more likely to accept an unfamiliar flavour later than would rats whose early gustatory experiences have been less varied This hypothesis has had some empirical support in the literature Kuo[1], for example, mentions pilot research suggesting that cats, dogs and mynah birds raised on restricted diets for

extended periods avoided new food, while others of their kind reared on varied diets ate novel foods readily We have now found a similar effect with rats using flavoured solutions rather than solid foods

The basic experimental plan (Table 1) was to expose mature and immature albino and hooded male rats (equally divided between ages and conditions) to either one or several flavours in the 'training' phase of the study Thus, for mature and immature rats alike, each of three groups was restricted to a single flavoured water to drink with its food for the 12 d of training, while a fourth group received all three of these flavours, one at a time, during the same period A further group of immature rats was given tap water during training The appropriate solutions and Charles River chow were available throughout the study After a 2-d interim of water following training, the rats were given a choice between tap water and a novel chocolate solution for 10 d During these two-bottle tests, as during training, the rats were never without food and drink except for the time it took each morning to weigh the animals, record the liquid consumed (to nearest 0 5 g) and refill the bottles to a common level (each holding more than the rat could consume in 24 h) A series of identical tests, lasting 6 consecutive days, was conducted a month after the initial block of tests, the rats being given tap water during the interim Individually caged, the rats were naive at the start of the experiment with respect to the training and testing flavours, which all consisted of 30 parts tap water to 1 part flavour by volume (All flavours were of an imitation variety (Kroger brand), except for the test flavour which was a pure chocolate extract) The solutions, given at room temperature were refreshed periodically The right-left position of the test bottles was changed after each day's weighing and refilling

The main results for each age category are given in Figs 1 and 2, which present the mean percentage of chocolate solution
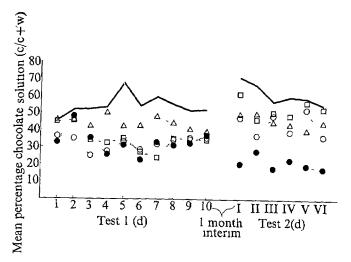

Fig 1 Mean percentage chocolate solution consumed by immature rats (10 per group) Mean percentages of chocolate drunk over all test days test 1, tap water (●) = 0 33, rum (□) = 0 35, black walnut (○) = 0 36, vanilla (△) = 0 44, varied (—) = 0 56 test 2, tap water = 0 21, black walnut = 0 44, vanilla = 0 45, rum = 0 53, varied = 0 62 (Symbols are the same for both tests) Mean amount (g) of solution consumed in training (given first) and test 1 black walnut (23 7, 46 7), rum (21 7, 49 9), vanilla (25 7, 44 2), varied (26 8, 49 6), tap water (24 8, 39 8) The experiment was repeated with immature rats using maple rather than chocolate A 30/1 solution of Kroger's imitation maple proved to be too palatable, with all of the groups, restricted and varied alike, drinking large proportions of the novel flavour When the concentration of maple was increased to 10/1 midway through the first test series, the curves separated, with the varied group holding at a high level of maple consumption and all of the restricted groups declining noticeably for the duration of the tests This illustrates the importance of using test flavours of only moderate to low 'natural' palatability so as not to obscure any training effects which might otherwise be there
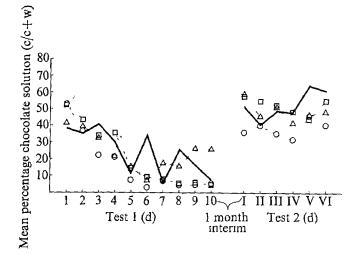

Fig 2 Mean percentage chocolate consumed by mature rats (nine per group) Mean percentages of chocolate drunk over all test days test 1, black walnut = 0 16, rum = 0 21, varied = 0 25, vanilla = 0 29 test 2, black walnut = 0 38, vanilla = 0 49, rum = 0 52, varied = 0 52 Mean amount (g) of solution consumed during training (given first) and test 1 black walnut (43 3, 51 8), rum (43 2, 49 5), vanilla (49 1, 46 7), and varied (49 8, 48 4) The symbols are as in Figure 1

consumed in each of the initial tests (1–12) and in the second series (I–VI) given 1 month later Individual scores were transformed (arc sin√preference ratio) before statistical analysis to normalise the distribution Basic to the design was our belief that relative consumption of the novel solution would be greatest by the 'varied group' trained on all three flavours The prediction was borne out for the immature rats (Fig 1) analysis of results for the first series of tests showing a significant training effect across training conditions $(F = 2 72, d f = 4,45, P < 0 05)$ but not over blocks of test days $(F = 1 33, d f = 4, 180)$ A significant training effect allowed us to compare individual training conditions over all days in test 1 by a Newman–Keuls procedure[2] All restricted groups, including the water controls, drank significantly lower percentages of chocolate solution than did the rats trained on three flavours $(P < 0 05)$ If a percentage of 0 50 or higher indicates a preference for chocolate, then only the varied group showed a slight to moderate preference for this novel flavour on several days of test 1 This is reflected in the overall averages reported at the bottom of Fig 1, with the highest mean percentage of 0 56 in test 1 for rats trained on a variety of flavours The intermediate mean of 0 44 for the vanilla group was significantly different $(P < 0 05)$ from the water control (0 33), but not from levels reached by the other restricted groups (rum = 0 35 and black walnut = 0 36) This suggests that vanilla and chocolate taste more similar to rats than do any of the other training flavours and chocolate

Results of the second series of tests for immature rats underscore the earlier finding, that is, relatively greater consumption of chocolate solution by the group that experience diversity of flavours early in life $(F = 3 39, d f = 4,45, P < 0 05)$ A significant main effect was found over blocks of test days $(F = 6 36, d f = 2,90, P < 0 01)$, with individual comparisons showing the varied group to have drunk significantly higher percentages of chocolate solution on days I–IV than its restricted counterparts $(P < 0 05)$ In addition, all curves for the flavour-trained groups were well above the level reached by the water control rats for the entire test period $(P < 0 01)$ This uniformly low consumption of chocolate in the control condition may reflect the impact of even a modest degree of diversity (be it a single training flavour) on later retention or augmentation of such preferences

The results for the mature rats (Fig 2) are unlike those for the immature subjects Most importantly, these groups of rats trained at an older age did not differ from one another in test 1

($F = 1 22$, d f $= 3,32$), though they all showed, more or less, a steady decline in percentage of chocolate consumed over the 10-d test period ($F = 28 21$, d f $= 4$, 128, $P < 0 01$) When retested a month later, the chocolate consumption of all groups increased from that at the conclusion of test 1, approaching levels attained by rats trained when immature (comparison of test 2 in Figs 1 and 2) As in the initial tests, however, the mature groups did not differ from one another in test 2 ($F = 1 87$, d f $= 3,32$) in a manner consistent with our hypothesis The mature rats, as a whole, more than doubled their mean percentage intake of chocolate solution from test 1 to test 2 (from 0 23 to 0 48), while the immature rats increased much less (from 0 41 in test 1 to 0 51 in test 2)

Finally, the average daily amounts of flavoured solution consumed by all groups during the training and first testing phases of the experiment are presented in the captions of Figs 1 and 2 For the immature rats, there seems to be a slight positive relationship between the amount of training flavour consumed and the percentage of chocolate drunk in testing, with the vanilla and varied groups ingesting the most chocolate and the most training solution But although these two groups drank similar amounts during training, the varied group consumed proportionately more of the chocolate during both test periods than did the vanilla group

Table 1 Summary of experimental design and procedure

Age (d)						
(Immature)	25–36	37–38	39–48	49–79	80–85	
(Mature)	65–76	77–78	79–88	89–119	120–125	

Group	n	Training	Interim	Test 1	Interim	Test 2
Restricted	10/9	Black walnut	T a p w a t e r	24-h choice of novel chocolate solution or tap water	T a p w a t e r	24-h choice of chocolate solution or tap water
Restricted	10/9	Rum				
Restricted	10/9	Vanilla				
Varied*	10/9	All three				
Control†	10	Tap water				

*The flavour solutions for this group were varied every 2 training days, such that some rats received black walnut for the first 2 d, then 2 d each with rum and vanilla, others were given rum, vanilla and black walnut, and still others, vanilla, black walnut and rum, each order repeated for the duration of training Each of the other groups was restricted to a single flavour (either black walnut, rum or vanilla) or tap water for the entire training period

†This condition was included after the main study was completed It serves as an additional comparison group (the most restricted of all) from which to gauge the effects of training A comparable group of mature rats was not used because of the general ineffectiveness of training for these animals

‡A 'novel' flavour is operationally defined here as one to which the animal had not been exposed before Of course, it may well taste like something the rat had had, and this is the reason for the design used here Since all the flavours experienced by the varied group are represented in separate experimental conditions any differential effects found relating to the dimensions of diversity and restriction should reflect something more than a simple generalisation (or summation of generalisation) from training flavour(s) to test flavour(s)

Our findings show that young rats will accept an unfamiliar flavour more readily if their early taste experiences are varied rather than restricted Furthermore, the effect seems to persist into maturity, at least for the conditions described here A comparable effect was not found for rats who were older at the start of training These rats did show, however, a dramatic decrease in chocolate consumption during test 1 and a subsequent (sustained) increase in test 2 Though we have no satisfactory explanation, a commonsense account of these results suggests simply that the older rats, treated as they were in this experiment, first tried chocolate, tired of it after a day or so, and then rebounded a month later because of their earlier (non-aversive) exposure to it Obviously this is more description than explanation and additional research is needed before more can be said

Our results suggest something different from what is

ordinarily implied by the concept 'stimulus' (that is, 'taste') generalisation, or the related concept of 'summation of generalisation' This is we believe for two reasons first, the effect was found only with immature rats (thus it is age-dependent), and second, none of the flavour-restricted immature rats, especially the water controls, reached the high levels of chocolate consumption shown by those trained on all three flavours—either during the immediate tests or a month later In some sense, more flavours were familiar to these latter animals than to their more gustatorily sheltered counterparts This is in keeping with an interpretation based on the notion of 'transfer of diversity', whereby the animal's earlier experiences (maybe at some 'sensitive' period in life) either do or do not prepare it for diversity or unfamiliarity later on[3,5]

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Independence of channels in colour contrast perception

IN the perception of brightness the responses from all three classes of cone in the human eye are pooled Evidence from recent electrophysiological measurements[1], however, indicates that the contrast detected by a particular colour channel is assessed independently of the summation Specifically, the existence of a red sensitive channel in which this occurred was demonstrated

In the course of experiments concerned with the effectiveness of displays for use in aircraft cockpits[2,3] we have compared the performance of subjects viewing red and green displays in a background of very high illuminance (10^5 lx) The results of these experiments, conducted in very different conditions at a much higher illuminance, support the suggestion of at least one visual colour channel in which contrast is assessed to some extent independently of other channels These results also have important implications in the field of display design

Each subject read two sets of 36 numbers and letters (each set forming the complete alphabet plus the numbers 0 to 9) presented singly in random order The vertical dimension of the characters subtended $0 3°$ at the eye A complete set in one colour was read before the colour was changed Alternate subjects saw the colours in the reverse order and the results were averaged in order to eliminate learning or fatigue effects The characters were back projected on to a small aperture in a screen in the high illuminance apparatus described previously[2] The screen is white in the upper half plane and black in the lower and thus simulates the conditions of an aircraft cockpit Each character had a luminance of 100 cd m⁻² and was presented for $0 7$ s Care was taken to balance the luminances of the red and green displays (peak wavelengths 660 and 560 nm respectively) and this was achieved to within 17%

More than 120 subjects participated in the experiment, of whom 68 (55 male, 13 female) were free from visual defects The results for these 68 subjects, expressed as an average error rate per subject are shown in Table 1 from which it is evident that the error rate for the green display was approximately

Table 1 Error rates for reading characters of different colours

	Error rate (%)	Omission rate (%)
Red display	5 8	1 1
Green display	15 9	5 6

Relative error rates for 68 observers (average age 31 5 yr) without visual defects, viewing red and green displays of equal luminance in an illuminance of 10^5 lx. The error rate includes characters wrongly identified and those for which no identification was offered (omissions)

3 times that for red This finding is statistically significant at the 0 05% level

Since the red and green displays were of equal luminance and the background illumination was the same for both, the contrast, considering total radiation, was the same for each display Thus the large difference in error rates found in this experiment is consistent with the existence of one or more colour channels in which contrast is assessed independently of the assessment of the overall brightness

We have estimated the ratio of the contrast in the red channel to that in the green channel although such calculations involve considerable uncertainties Two approaches were used For the first, we used the data of Wald[4] for the action spectra of the red and green cone pigments These data are supported by later results[5] which give similar ratios (within 25%) of red to green cone response over the range of measurement (540-640 nm) Although the response to the red display was found to be exclusively associated with the red cones, the response to the green display was divided fairly evenly between the red and green cones (42 58) The effect of the background illumination was somewhat higher for the red cones (56 44) A comparison of the response of the red cones to the red display with that of the green cones to the green display, allowing for the different responses of these receptors to the background, showed the contrast in the red channel for the red display to be higher by a factor of 1 35

The second estimate was based upon the high intensity colour mechanisms of Stiles[6] and this calculation showed the contrast for the long wavelength mechanism π_5' when the red display was viewed to exceed that of the π_4' mechanism viewing the green display by a factor of 1 80

Both of these estimates seem to be rather low to account for the large difference in error rates They should be regarded as tentative, however, reflecting the difficulties of obtaining separate spectral responses for colour vision receptors as well as differing experimental conditions

These considerations do not affect the conclusion that the large difference in error rates is consistent with the independent assessment of contrast in one or more colour channels and which therefore lends support to Regan's findings Similar conclusions are emerging from response time experiments at present being conducted in this laboratory[7]

A complete account of these and related experiments including details of the variation of performance with age, display format and character fount, is in preparation It is, however, worth mentioning that the consequences of the results given here for cockpit displays are of some significance There is a popular belief that green displays are likely to be superior to red "because the eye response peaks in the green" Our results show that in very high ambient illumination this belief is ill founded and it is more difficult to make an effective green display.

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Inhibition and disinhibition of direction-specific mechanisms in human vision

MOTION-SENSITIVE mechanisms in human vision are selective for direction of stimulus movement Psychophysical experiments reveal direction-specific channels which can be selectively desensitised or adapted[1,2] At threshold these direction-specific mechanisms operate independently, they have little or no sensitivity for the opposite direction of motion[3-8] Independence at threshold does not, of course, preclude interaction at suprathreshold stimulus levels Indeed, inhibition has been found above threshold between otherwise independent spatial frequency-specific mechanisms[7,8], orientation-selective mechanisms[9,10], and binocular disparity-specific mechanisms[11] We report here comparable measurements of inhibition between direction-specific channels We also show that in appropriate conditions the inhibition can itself be reduced or eliminated, a disinhibition effect

To search for direction-specific inhibition at suprathreshold levels, we used a composite adaptation method[7,8] The term 'adaptation' refers only to the elevation of detection threshold produced by exposure to an appropriate high contrast stimulus, we make no assumptions as to the processes underlying adaptation[12,13] Contrast is defined as the peak-to-trough luminance amplitude of a grating divided by twice the average luminance Stimuli were vertical sinusoidal gratings created on a cathode ray tube, using a television technique[14] (10° square raster, average luminance 3 4 cd m^{-2})

Thresholds for a moving grating were measured following adaptation to either a high-contrast grating (same spatial
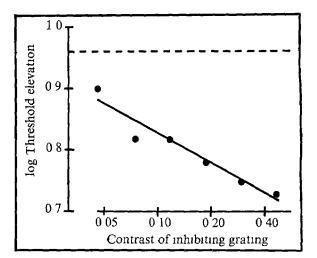

Fig 1 Threshold elevation (1 75 cycles per degree, 7 9 Hz) for a rightward-moving test grating as a function of the contrast of a leftward-moving inhibiting grating Contrast is specified separately for each component of the composite adaptation stimulus Zero log threshold elevation represents the threshold for the rightward test grating after simple light adaptation (a zero contrast adaptation stimulus) The dashed line indicates threshold elevation produced by the rightward-adapting component alone Each point is the mean of six measurements (s e < 6%) The slope of the solid line (fitted by least squares) is significantly less than zero ($P < 0 005$) Gratings were made to drift using motor-driven synchro-resolvers[15] Viewing was monocular and foveal, fixation was carefully maintained A session began with 3 min continuous exposure to an adaptation stimulus Every 4 s thereafter the adaptation stimulus was replaced for 1 s by a test grating of variable contrast The observer adjusted a potentiometer to reduce the contrast of the test grating until it was just indistinguishable from a uniform field of the same average luminance

frequency and drift rate as the test) moving in the same direction as the test grating, or the same high-contrast grating to which had been linearly added another grating, moving in the opposite direction An adapting stimulus which drifts in the same direction as a test grating (that is, the first type of adaptation stimulus) elevates test threshold, an adaptation grating which moves in the opposite direction (when presented alone) also elevates test threshold, although the amount of elevation is considerably less than is produced by a same-directional grating of equal contrast[1,2] One might expect these individual adapting effects to sum when a composite adaptation grating (the second type of adapting stimulus) is used We have found, on the contrary, that addition of the opposite-directional grating gives less threshold elevation than does the same-directional grating alone This constitutes an inhibitory effect
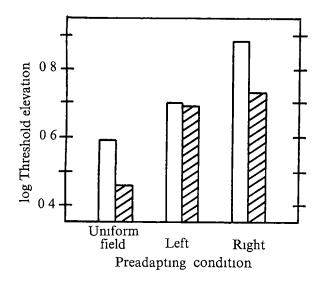

Fig 2 Demonstration of disinhibition Threshold elevation (1 75 cycles per degree, 7 9 Hz) for a rightward-moving test grating produced by adapting to rightward movement alone (open columns) or to rightward plus leftward movement (hatched columns) is shown separately for each preadapting stimulus The bars are means of 12 measurements, s e < 6%

Figure 1 shows the log threshold elevation for a test grating moving to the right as a function of the contrast of an inhibiting component (opposite direction) of the adaptation stimulus The contrast of the adapting grating drifting to the right was fixed at 0 23 As the contrast of the opposite-directional component moving to the left increases, threshold elevation is reduced The maximum inhibition effect is just over 0 2 log unit Increasing the contrast of the component moving to the left reduces the adapting power of the grating moving to the right The leftward grating seems to inhibit the activity of the rightward component Similar data have been obtained at other spatial and temporal frequencies and with a second observer The measurements have been verified under conditions of forced-choice testing as well

These results show that direction-specific mechanisms can inhibit one another We should also expect to see a related phenomenon, disinhibition, a reduction in the amount of generated inhibition when the inhibiting channel has itself been desensitised Disinhibition is generally regarded as occurring when the original inhibition-generating channel is itself inhibited by a third channel This is clearly impossible in the present experiment, since stimulus orientation must be held constant at vertical, and only two oppositely-tuned direction-specific channels may be considered Disinhibition by adaptation of the inhibiting channel, however, should produce the same result, and can provide at least as much information about the inhibitory process

To demonstrate disinhibition, we measured inhibition as above, but preadapted the inhibiting channel by exposure to its preferred direction of movement For a test grating moving to

the right, the inhibiting component of the adapting stimulus moves leftward, the preadapting grating, then, must also move to the left The direction-specificity of the adaptation process[1,2] ensures that the preadapting stimulus will desensitise the leftward, inhibiting channel much more than the rightward, adapting channel This should reduce the amount of inhibition exerted by the leftward mechanism on the rightward

Each adaptation period was divided into two intervals, each of 1 s duration and continuously alternating throughout the experimental session The first sort, a preadapting interval, was filled either with a leftward-moving preadaptation grating (contrast 0 46) or with an unmodulated field at the average luminance During the second, the inhibition interval, the observer viewed either the rightward-moving adapting grating (contrast 0 23) or the same adapting stimulus plus the additional leftward-moving inhibition grating (contrast 0 46)

When the preadaptation stimulus was a uniform field (Fig 2) a sizeable inhibition effect could be measured (about 0 13 log unit reduction in threshold elevation, $P < 0 001$, two-tailed t test) After preadaptation to the leftward-moving grating, however, no inhibition occurred ($P > 0 5$) To verify that this change did not result from the presence of any grating at all during the preadapting interval, we also preadapted to a rightward-moving (same-directional) grating (contrast 0 23), and obtained 0 16 log unit of inhibition ($P < 0 001$) Disinhibition seems to depend on preadaptation in the inhibiting direction (opposite to test direction)

This demonstration of disinhibition shows that the inhibition effect is not an artefact of nonlinear signal summation, either in the apparatus or in early stages of the visual system Across all three types of preadaptation stimulus shown in Fig 2, the configuration of the adapting and inhibiting gratings remained constant The only changes were in the adaptive states of the observer's direction-specific mechanisms Nevertheless, the inhibitory effect was eliminated in one condition Thus, the inhibition effect cannot result from physical peculiarities of the composite stimulus

Direction-specific neurones are known to be abundant in cat and monkey visual cortex[16-20] A direction-specific cell discharges strongly when stimulated by movement in one, preferred direction, the same cell fails to fire, or discharges at less than its maintained rate, when stimulated by movement in the opposite direction Such reduction in a neurone's response to movement in its non-preferred direction can repiesent an active inhibitory process[21-23] The inhibition may be generated by other cortical cells, with different direction preferences[24] The present psychophysical demonstration of inhibition between human direction-specific mechanisms is thus compatible with the hypothesis that human direction-specificity is mediated by directionally selective neurones in human visual cortex

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Genetic control of haploid parthenogenetic development in mammalian embryos

THE establishment of haploid cell lines in mammals would be of value for studies on developmental biology, genetics and carcinogenesis It should be possible to produce such lines by culturing cells from haploid parthenogenetic embryos Only a limited degree of haploid parthenogenetic development has been obtained with mammals[1-4], possibly as a result of the effects of deleterious recessive genes in haploid cells During the process of selection necessary to produce an inbred strain of animals, there may be a reduction in the number of deleterious genes Haploid embryos from random-bred animals that have not been subjected to the selection that occurs in inbreeding may, therefore, have a lower development potential than haploid embryos from an inbred strain To test this possibility, we have compared the development of haploid embryos from one inbred strain of golden hamsters with random-bred animals and have found better development of haploid embryos in the inbred animals

We used an inbred strain (LSH/SS) and random-bred golden hamsters, because embryonic hamster cells can be readily transformed into cell lines by various viral, chemical and physical agents[5,6] and their chromosomes can be easily identified Parthenogenetic development has been induced in the mouse by electrical stimulation of the oviduct after superovulation with gonadotrophins[3,4] This technique also induces a high frequency of parthenogenetic development in hamsters

The frequency of haploids and diploids was determined in parthenogenetically developing eggs at the single cell pronuclear stage, 10–12 h after electrical stimulation, and in embryos with more than two cells at 72–74 h after stimulation (Fig 1) About 60% of the eggs were activated in both the inbred and random-bred hamsters at 10–12 h as determined by the presence of one or two pronuclei At 72–74 h, about 30%

Table 1 Activation rate, egg development and haploid–diploid ratio, at 10–12 and 72–74 h after electrical stimulation of the oviducts of random-bred and inbred hamsters*

Time after electrical stimulation (h)	Random-bred or inbred animals	No of females	Total no of eggs examined	No of embryos Two-cell	No of embryos More than two-cell	% Activated eggs or embryos per animal† Cells with pronuclei	% Activated eggs or embryos per animal† Embryos with two or more cells	% Non-activated eggs per animal†, ‡	Haploid–diploid ratio§
10–12	Random	5	96	0	0	57 7 ± 10 2	0	13 5 ± 4 4	3 2 1
10–12	Inbred	5	189	0	0	59 6 ± 9 0	0	7 2 ± 3 4	1 8 1
72–74	Random	26	725	107	65	0	25 5 ± 4 5	34 1 ± 5 4	0 2 1
72–74	Inbred	11	349	81	47	0	33 5 ± 4 4	24 4 ± 5 5	1 7 1

* Females (7–12 week old) from random-bred and the LSH/SS inbred strain of golden hamster, were superovulated by intraperitoneal injections of 25 IU of pregnant mare's serum gonadotrophin followed 48 h later by 20 IU of human chorionic gonadotrophin (HCG) Females were anaesthetised with an intraperitoneal injection of 0 3–0 4 ml of a 24 mg ml^{-1} solution of Nembutal in physiological saline 19 5–21 h after the HCG injection

Eggs were activated *in situ* by electric shocks (50 V), as described for mouse eggs[3,4] The tips of two steel needles, which served as electrodes, were applied along the length of the ampullar region, and 20–25 shocks applied in rapid succession The animals were killed either 10–12 h or 72–74 h after treatment Their oviducts were removed and flushed with phosphate-buffered saline to collect the eggs or embryos Control groups without electrical stimulation were examined after superovulation and after Nembutal anaesthesia About 20% of these eggs from the controls developed pronuclei, but no two-cell embryos were observed When females were stimulated 15–16 h after HCG, only about 13% of the eggs isolated 72–74 h later were either two-cell or more advanced embryos Higher rates of activation were obtained when the oviducts were electrically stimulated 19 5–21 h after HCG, so that this time was used in the experiments Pronuclear eggs were cultured at 37 °C in NCTC 135 medium[10] with 10% foetal calf serum, under light paraffin oil in plastic Petri dishes in an incubator with 5% CO_2 in air The chromosomes of eggs were examined using an air-drying technique[11] in the first cleavage mitosis As with mice[12,13] all eggs which originally had a single pronucleus had a single haploid set of chromosomes, whereas those which originally had two pronuclei had a diploid first cleavage metaphase Cleavage embryos (8–9) beyond the two-cell stage recovered at 72–74 h after electrical stimulation, were examined after culturing for 2–3 h at 37 °C in phosphate-buffered saline containing 4 μg ml^{-1} colcemid The chromosome ploidy and cell number of these embryos was then determined The golden hamster has a normal diploid number of 44 chromosomes[14] Some 70% of the embryos with mitotic figures had two or more metaphase plates

† Eggs were only classified as activated if they had developed a pronucleus or had cleaved to the two-cell or later stage The percentages of activated and non-activated eggs or embryos per animal are given as the mean ± s e

‡ In the 72–74 h groups there were two classes of eggs Those which progressed to the first cleavage mitosis but failed to cleave to the two-cell stage, and those which were not activated These two classes could not be distinguished In all the experimental groups there were 30–40% degenerated eggs

§ In the 10–12 h groups, the ratios were determined both on the number of pronuclei present, one or two, and on subsequent chromosome counts The ratios in the 72–74 h groups were determined by chromosome counts on embryos that were more advanced than the two-cell stage In the 72–74 h groups, the ploidy could not be determined in 50% of embryos from the random-bred and 37% of the inbred animals, because there were no dividing cells

Table 2 Number of cells in embryos that had developed beyond the two-cell stage at 72–74 h

Random-bred or inbred animals	Haploid No of embryos	Haploid No of cells per embryo*	Diploid No of embryos	Diploid No of cells per embryo*	Embryos without cells in mitosis No of embryos	Embryos without cells in mitosis No of cells per embryo*
Random	4	7 0 ± 1 3 (4)	19	7 4 ± 1 1 (19)	23	7 1 ± 0 8
Inbred	17	6 8 ± 0 7 (17)	10	10 7 ± 1 2 (10)	16	5 7 ± 0 8

* Mean ± s e
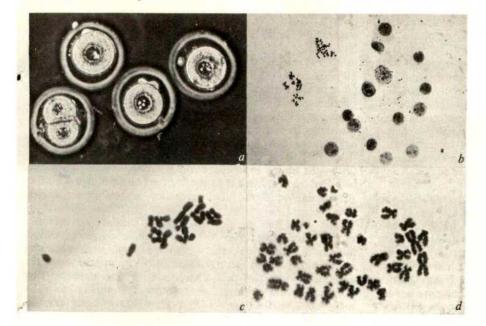Numbers in parentheses are numbers of embryos

Fig. 1 *a*, Parthenogenetically activated eggs from a random-bred hamster: three single-cell eggs with one pronucleus and one embryo at the two-cell stage 16 h after electrical stimulation ($\times$ 350). *b*, Diploid 16-cell parthenogenetic blastocyst from a random-bred hamster 72 h after electrical stimulation ($\times$ 250). *c*, Haploid metaphase plate from an 11-cell parthenogenetic morula from a random-bred hamster 72 h after electrical stimulation ($\times$ 1,500). *d*, Diploid metaphase plate from a 20-cell parthenogenetic blastocyst from an inbred hamster 72 h after electrical stimulation ($\times$ 2,500).

of the embryos were either at the two-cell or a more advanced stage. At both times there were 30–40% degenerated eggs. Since the proportion of degenerated eggs was not higher at 72–74 than at 10–12 h, this suggests that 30% of the activated eggs found at 10–12 h after stimulation failed to develop to the two-cell stage in both groups of animals and were indistinguishable from non-activated eggs. Although in previous studies with golden hamsters parthenogenetic development did not occur beyond the two-cell stage[7-9] we observed some well developed blastocysts with about 20 cells.

The ratios of haploids to diploids in the random–bred and inbred animals were 3.2:1 and 1.8:1 in the eggs at 10–12 h, and 0.2:1 and 1.7:1 in the embryos at 72–74 h, respectively (Table 1). The haploid to diploid ratio of eggs and embryos was, therefore, similar in inbred hamsters, with more haploids than diploids. In the random-bred animals, the ratio of haploid to diploid eggs was similar to that found in the inbred hamsters, but there was a much lower ratio of haploid to diploid embryos. The results indicate that in the random-bred animals, there was a block in more than 90% of the haploid eggs, so that they did not develop beyond the two-cell stage, but not in the inbred strain of hamsters (nor was there a block in two inbred strains of mice[3,4]). The average number of cells in embryos which had developed beyond the two-cell stage at 72–74 h, was about the same in both haploids and diploids (Table 2).

These results indicate that the development of haploid parthenogenetic embryos can be genetically controlled. The better developmental potential of haploid embryos from the inbred compared to the random-bred animals presumably results from a reduction in the number of deleterious genes occurring during the process of selection associated with inbreeding.

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Polymer exclusion, cell adhesion and membrane fusion

FROM physicochemical studies on the adhesion of fibroblasts to synthetic substrata[1,2] I suggested that, in general, surface-bound polymers (for example adsorbed serum proteins) probably inhibit cell attachment because of their steric exclusion volume. This effect is well known in colloid science (Faraday used gelatin to stabilise gold sols[3]) but does not seem to be widely appreciated in cell biology. Thus, a recent review on the role of proteins and divalent cations in cell adhesion, viewed as a problem in colloid stability, discusses only charge repulsion and calcium bridging[4]. Furthermore, standard texts on the cell surface[5] present the classical DLVO (Derjaguin, Landau, Verwey and Overbeek) theory of charge repulsion against dispersive attraction, but omit the later development by Overbeek and coworkers of the principle that even uncharged surfaces, if covered by adsorbed polymer molecules, may repel each other[6]. I now show that steric exclusion by glycocalyx polymers on the plasma membrane would explain some recent observations on fusion and attachment, by a variety of cells. The term steric exclusion[3] comprises not only geometric exclusion by rigid molecules, such as rods and spheres, but also unfavourable thermodynamic parameters such as free energy, summed up over many segments, and, for random coil polymers, entropy resulting from the ability of each molecule to claim extra lebensraum by flexing its segments.

This model predicts mutual repulsion between two closely apposed membranes, because of the exclusion volumes of their respective glycocalyx macromolecules. Conversely, fusion should be facilitated if glycopolymers are cleared from between the two membranes, by lateral displacement, to appose the naked lipid bilayers. Such a mechanism of fusion, by 'the interaction and mixing of the disturbed lipid molecules of closely apposed membranes in regions denuded of intramembranous proteins and glycoproteins' has been proposed[7]. The steric exclusion model, developed independently from studies on cell adhesion (S. P. Pegrum and N.G.M., unpublished), provides a general physicochemical basis for these observations on fusion. For instance, in discussing the

role of cryoprotective solvents in the lateral aggregation of intramembranous particles, Ahkong *et al.*[7] concentrate on lipid effects, but exclusion implies interaction with the proteins of membranes. In terms of colloid chemistry, regarding the glycocalyx polymers as hydrophilic sols, such dehydrating solvents would be expected to cause their flocculation (for example, ref. 8). Moreover, the excluded volume for flexible polymers can be shown on general theoretical grounds[9] to decrease in poor solvents, because of the increased preference for segment–segment contacts over segment–solvent interactions. (In fact the lateral aggregation of polymer molecules on a fluid interface may be a convenient method for the study of such interactions.)

A different aspect of cell adhesion which suggests the importance of avoiding fusion is based on the observation that cells do not adhere to pure, solid hydrocarbon[1]. These experiments were originally carried out in serum, but have been extended to a protein-free medium[2], so that steric exclusion is not then a factor. In this medium cells attach and spread on condensed, rigid substrata with critical surface energy greater than 43 dyne cm^{-1} (ref. 1) that is, those presenting pure amide, hydroxyl, ester and acid groups—so why not methyl and methylene? At first sight the inertness of hydrocarbon might seem to lack biological relevance—it is well known that solid paraffin is not a natural substratum for cell adhesion. This result seems remarkable if one recalls that cellular tissues contain high concentrations of almost pure hydrocarbon, both solid and liquid, near the front line of adhesion—in the lipid bilayer. I conclude that, contrary to the basic postulate of DLVO theories for cell adhesion[5], the cell does not rely on non-polar, lipid–lipid interactions, because such interactions would lead to an increased probability of fusion between the hydrocarbon interiors of apposing lipid bilayers. Hence I postulate a model in which the mechanism of cell adhesion starts with two lines of defence against spontaneous and indiscriminate fusion of membranes—the first, steric exclusion by glycocalyx polymers; the second, reliance on polar, short range attractions rather than long range, non-polar dispersive forces.

Steric exclusion would also explain the low adhesivity of neutral, hydrophilic gels, such as agarose[1] or cross linked serum[10]. In a previous comparison of the supramolecular structures of agarose and collagen, I attributed the adhesivity of the latter both to the greater mechanical rigidity of its fibres and to their more substantial molecular packing[1]. Subsequent experimental data has emphasised the importance of molecular aggregation. With or without serum, adhesion to rigid agarose is greatly improved if the latter is first annealed, near its setting temperature, to produce dense, granular microaggregates; this method of clustering double-helical agarose molecules in close side-by-side association was suggested by Dr D. A. Rees (N.G.M., unpublished). Similarly, Gordon and Dingle found that preincubation of soluble collagen, to form submicroscopic fibrils, increases its binding by platelets in serum-free buffer[11]. I have found that increasing the concentration of a hydrophilic gel (sauflon polyvinylpyrrolidone; PVP) from 40% to 96% renders it adhesive for fibroblasts. These observations on the role of molecular clustering relate to preliminary reports, by Lyman and coworkers, that both thrombocytes and fibrocytes react to some microarchitectural or 'tacticity' difference between homologous copolymeric substrata[12].

They also suggest an answer to the problem of how cells overcome the exclusion barrier and adhere to polymer-coated surfaces. Divalent cations[4,13] and bridge-forming 'adhesion factors'[14] have been implicated, but longitudinal, that is, surface-to-surface, bridging does not explain one widespread feature of cell adhesion—the presence of dense, localised 'plaques'[15] and desmosomes. These structures imply a role for lateral bridging as well. Cells fixed by cross-linking with aldehyde (and thus, I presume, with their membrane proteins intact but incapable of lateral movement) bind more weakly, whether to collagen directly[11] or to each other by way of a bridging factor[14]. Lateral aggregation ('patching') is readily induced by lectin-like or antibody-like factors, in practice; but hitherto there has been no general reason to expect this as a feature of cell adhesion, in principle. The theory of excluded volume seems to provide a reason for the cell to concentrate glycocalyx polymers at the point of adhesion, because the excluded volume decreases as molecular crowding increases[9]. Since a large excluded volume depends on the possibility of molecules flexing[6], the formation of dense lateral aggregates in the glycocalyx would restrict this effect, especially if matched by a corresponding clustering of the substratum (as in the above experiments on hydrophilic gels). Thus the cell may require divalent cations not only as a bridge with the substratum but also for the aggregation of membrane particles into plaques.

This raises the question of adhesive interactions between plaque and substratum. Although positive charge improves adhesivity[10], the wide variety of non-physiological substrata, both negative[2] and neutral (agarose, PVP) implies bonding additional to that of fixed charge, on the one hand, or biochemically specific groups, on the other. Preliminary electron-microscope studies (with S. P. Pegrum) show direct contact and incipient fusion between membrane and substratum, in protein-free medium. These observations are more in agreement with Zisman's theory of adhesion as a process akin to wetting[1,2] and thus additionally subject to close-range but nonspecific forces, such as hydrogen bonds and hydrophobic bonds.

I conclude that a model of cell adhesion must possess the following general features, which depend on physical chemistry, in addition to biological specificity of the sort found in antibodies, lectins and enzymes: minimal attraction between the hydrocarbon interiors of two apposing membrane bilayers; preference for solid, polar substrata; membrane glycopolymers inhibit adhesion and fusion through their exclusion volumes; exclusion effect countered by dense, local aggregates in glycocalyx (as in hydrophilic gels); this allows 'adhesion as wetting', that is, through short range but nonspecific bonds. A model cell based on these principles would be architecturally both stable (because the first three protect crowded membranes from spontaneous fusion and indiscriminate adhesion) and flexible (because the other two allow localised attachment to a variety of substrata).

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Possible involvement of the plasma membrane in saltatory particle movement in heliozoan axopods

THE suggestion that saltatory particle movements in heliozoan axopods, melanophores, nerve axons, as well as in other less specialised cells, are mediated by microtubules has been tendered[1-5]. This notion is based on morphological observations, and experiments demonstrating the cessation or alteration

of movement in response to colchicine, a drug which destroys microtubules[3,6-8]. Recent evidence, however, does not favour microtubules as the immediate driving force for such systems[9-10].
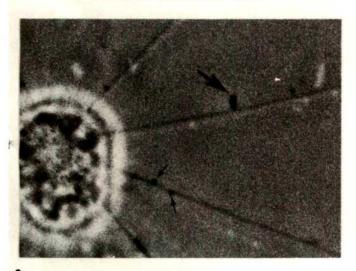
Heliozoans are suited to studies of intracellular movement, because the kinetocysts[11] which shuttle back and forth within their axopods do so with a readily definable sign and path. The axopods contain a straight rigid core of microtubules[11,12]. This report analyses the movement of kinetocysts in a heliozoan, and of bacteria, themselves non-motile, which occasionally adhere externally to the axopods. The results suggest that the plasma membrane, rather than the axonemal microtubules, may be involved.

Heterophrys sp., a small marine centrohelidian, was filmed at 10.25 frames per second At five frame intervals the positions of continuously moving kinetocysts and bacteria were determined. The points obtained were tested for goodness of fit to a regression line[13] of the form $y = a + bx$, where y represents particle displacement, a elevation, and x time. The regression coefficient b is then the best measure of particle velocity. To obtain enough points for an analysis of each displacement, only those movements which lasted at least 20 frames (1.8 s) were considered. The direction of movement, the distance traversed (d), and the approximate distance (D) of the particle from the cell body at the start of movement were also recorded.

Almost all of the displacements fitted a linear regression at the 5% significance level, indicating that, once in motion, the particles move with uniform velocity and thus their movements are clearly saltatory. The regression coefficients, however, ranged from 0.4 to 2.8 µm s^{-1} ($n = 47$), demonstrating that the speed varies strongly between displacements. Since b for several displacements of the same particle also frequently varied significantly, the velocity of a given particle cannot be related simply to its mass or density.

Comparison of the average velocities of centripetally ($b_{in} = 1.09 \pm 0.11$ µm s^{-1}, $n = 25$) and centrifugally ($b_{out} = 0.64 \pm 0.05$ µm s^{-1}, $n = 13$) moving kinetocysts showed that these were significantly different ($0.01 < P < 0.05$). The Behrens–Fisher test was used because the variances were found to be significantly different in an F test ($P < 0.001$). A one-sided hypothesis permits the conclusion that kinetocysts move faster inward than outward ($P < 0.025$). A correlation test and a regression analysis showed that b_{in} and the distance moved

toward the cytosome (d_{in}) are significantly positively correlated ($r = 0.6516$, d.f. = 23, $P < 0.001$) and fit well the expression $d_{in} = -1.10 + 3.72\ b_{in}$ ($P < 0.001$). Thus the faster the kinetocysts move toward the cell body, the longer are the distances traversed. No such correlation could be shown for centrifugally moving kinetocysts ($r = -0.4216$, d.f. = 11, $P > 0.1$). Additional correlation tests demonstrated that b_{in} and b_{out} and d_{in} and d_{out}, are not significantly correlated with D. Therefore, the velocity and path lengths of moving kinetocysts are independent of their position along the axopod at the start of saltation. Finally, tests of the null hypotheses, H_0: $d_{in} = d_{out}$ and H_0: $D_{in} = D_{out}$, were not significant. This supports the experimental technique, because they do not demonstrate a bias in the selection of inwardly and outwardly saltating particles with respect to their average path length or initial distance from the cytosome.

Although the data obtained for saltating bacteria were few, a significant correlation of b_{in} with d_{in} could be demonstrated ($r = 0.8890$, d.f. = 3, $0.02 < P < 0.05$). The expression for the regression of path length on velocity was $d_{in} = -1.08 + 4.42 b_{in}$ ($P < 0.05$). A correlation test of b_{out} and d_{out} was not significant ($r = 0.9019$, d.f. = 2, $P > 0.1$). Thus the kinetocyst and bacteria saltatory movements seem to be similar in character, both showing correlation of b_{in} with d_{in}, but not of b_{out} with d_{out}. The regressions of d_{in} on b_{in} for kinetocysts and bacteria were compared in an analysis of covariance[13]. No significant differences between their residual variances ($F = 0.72$, d.f. = 23, 3), slopes ($F = 0.22$, d.f. = 1, 26) or elevations ($F = 0.70$, d.f. = 1, 27) were found, showing that the regression lines are not significantly different.

The different average velocities and variances of centripetally and centrifugally moving kinetocysts, and the correlation of velocity with path length during inward, but not outward movements of both kinetocysts and bacteria, imply that two fundamentally different mechanisms are responsible for transport to and from the cell body. A similar situation exists in melanophores where aggregation and dispersion are known to be differentially sensitive to drugs[14,15]. The correlation of b_{in} with d_{in} also suggests that centripetal movement in axopods is an ordered process with elastic properties reminiscent of the snapping of a rubber band; the more the band is stretched before it is released, the faster and farther something attached to it will be moved. Centrifugal movement, in contrast, is unordered and wholly inelastic.

The microtubules of the axoneme can hardly be involved in saltation, for when they are destroyed by colchicine, similar movement continues, although not in straight lines (ref. 16 and D.T., unpublished). Since the kinetocyst and bacteria centripetal movements are statistically indistinguishable, it is possible that the forces motivating both are the same. The kinetocysts, however, move under the plasma membrane, whereas the bacteria are clearly extracellular, exhibiting unmistakable Brownian motion in the surrounding medium, especially when adherent at only one end (Fig. 1). If both types of particle are moved by the same mechanism, the possibility cannot be ignored that the probable site of force application is the plasma membrane itself, the only structure in common contact with kinetocysts and bacteria. Speculation that the membrane is involved receives support from a recent demonstration that the rate of area change and initial area of a triangle of plasma membrane delimited by three extracellularly adherent gold particles are correlated when the particles aggregate, but not when they disaggregate[17]. In other words, aggregating particles on the membrane move with precisely the same elastic qualities as shown here for centripetally saltating structures; particle disaggregation, like centrifugal saltation, is unordered.

Whether movement in axopods involves gradual autonomous stretching and subsequent elastic release of the membrane itself, or an interaction between membrane islands[17] or anchorages[18] and extrinsic contractile protein remains to be seen. That actin[19] and myosin[20] exist in other cells in an appropriate position to be capable of causing the kind of membrane-

Fig. 1 Frame from a film of *Heterophrys* sp. A bacterium (large arrow) adheres at its end to an axopod. The small arrows indicate kinetocysts. Phase contrast, ×3,400.

associated movement described here has already been shown. *Heterophrys* sp. was provided by Dr M. Hauser.

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Tryptic destruction of aggregative competence in *Dictyostelium discoideum* and subsequent recovery

WHEN they reach the stationary phase, the vegetative cells of *Dictyostelium discoideum* become adhesive and form multicellular aggregates before forming fruiting bodies. In shaken liquid suspension they form aggregates in two stages[1]. First,
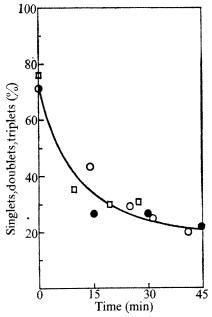

Fig. 1 Aggregates at the 15 h stage of fruiting body construction were harvested from filters in 17 mM K/Na$_2$ phosphate, pH 6.8, mechanically dispersed to a cell suspension by repeated forceful pipettings through a 10 ml pipette, and diluted to a density of 10^7 cells ml^{-1} in phosphate buffer containing the following additions: ○, no additions, ●, 2% v/v glycerol, 10 mM EDTA, 0.5 mg ml^{-1} type III trypsin (T, Sigma), 0.5 mg ml^{-1} type 1 S trypsin inhibitor (T I, Sigma); □, all of the previous additions plus 0.5 mg ml^{-1} cycloheximide (Sigma). Volumes (20 ml) of suspension in 150 ml Erlenmyer flasks were incubated at 22 °C on a rotary shaker at 110 r.p.m. At intervals samples were examined in a haemocytometer to determine the distribution of cells as either singlets, doublets, triplets or larger aggregates. A significant reduction in the first category was accompanied by an equivalent increase in the second.

they produce loose amorphous clusters which, if deposited on a solid substratum, immediately disperse. In the second stage (6–7 h) the clusters have become tight, round or ovoid aggregates eventually covered with a thin cortical sheath. If deposited on solid substratum, they remain intact and immediately proceed to the next stage of fruiting body construction. The loose clusters are EDTA-sensitive, the tight aggregates are not[2].
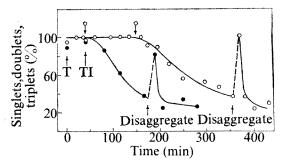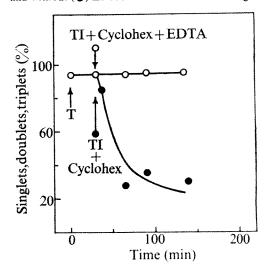

Fig. 2 Cells were collected at 15 h culture, dispersed and diluted to a density of 10^7 cells ml^{-1} in phosphate buffer containing glycerol, EDTA, and trypsin. After 40 min, trypsin inhibitor was added with (○) and without (●) cycloheximide (first arrow). Cycloheximide was removed (second arrow) from the former by centrifuging the suspension for 10 min at 400g in a Sorvall swinging bucket rotor, washing twice in phosphate buffer containing glycerol and EDTA and resuspending the cells in 20 ml of that solution. The incubation was then resumed. When noted, aggregates were dispersed by repeated pipettings, as before to measure the kinetics of reaggregation. Concentrations as in Fig. 1.

Aggregated cells can be mechanically dispersed to yield a single cell suspension which if shaken gently reforms tight aggregates within a few minutes. The experiments to be described demonstrate that, first treatment of disaggregated cells with trypsin destroys their capacity to form tight EDTA-resistant aggregates without affecting their capacity to adhere in loose, EDTA-sensitive clusters; second, on continued incubation after removal of the trypsin by inactivation, the cells slowly reacquire aggregative competence but cannot do so in the presence of cycloheximide; and third, the initial aggregative competence, its destruction after trypsin treatment, and its subsequent reacquisition are precisely reflected in the adhesive properties of ghosts derived from the untreated and treated cells.

Figure 1 shows the kinetics of reaggregation by freshly disaggregated cells. Confirming the earlier finding[2], the process is EDTA-resistant. It is unaffected by trypsin already inactivated

Fig. 3 Trypsin treatment was carried out as before but in the absence of EDTA. At 10 min intervals the reclustered cells were redispersed by repeated pipettings. After 30 min, trypsin inhibitor and cycloheximide (cyclohex) were added with (○) and without (●) EDTA. Concentrations as in Fig. 1.

by trypsin inhibitor, by glycerol (present to provide osmotic protection against trypsin) and by cycloheximide. In contrast cells treated with trypsin for 40 min before the addition of trypsin inhibitor reaggregated very slowly (Fig. 2) but after about 2.5 h had regained full EDTA-resistant, aggregative competence. The presence of cycloheximide prevented this reacquisition but the inhibition was shown to be reversible after removal of the drug. Clonal platings demonstrated that cell viability was unaffected by any of the treatments employed.

Disaggregated cells treated with trypsin in the absence of EDTA continually reclustered and had to be kept apart by frequent mechanical disruption. If after the trypsin was inactivated, EDTA (and cycloheximide) were added, the cells remained dispersed (Fig. 3). In the absence of EDTA, however, loose clustering was immediately resumed. Thus, trypsin treatment destroys only the second-stage adhesive capability and does not affect the first.

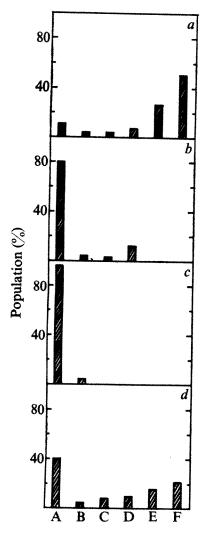Recently it was demonstrated that ghosts derived from pre- and postaggregative cells retain the adhesive behaviour of the cells from which they were derived. A cofactor requirement for aggregation could be satisfied by addition of any of a specific group of divalent cations (Mn^{2+}, Ca^{2+}, Zn^{2+} and Cu^{2+})[3]. Figure 4 shows that in the presence of Ca^{2+} and EDTA, ghosts prepared from disaggregated cells, from trypsin-treated cells and from cells allowed to recover from trypsin treatment in the presence and absence of cycloheximide reflected precisely the adhesive capacities of the cells from which they were derived. This provides assurance that the trypsin-mediated lesion is located in the plasma membrane and is intimately associated with the specific adhesive sites. We are attempting to determine what is removed from the membrane by trypsin treatment and what is replaced during the recovery period. It will also be interesting to determine the relationship, if any, between these tryptic lesions and the antigenic determinants on the membrane which accumulate during the preaggregative period and which have been implicated in the adhesive process[4,5].

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Fig. 4 Untreated control cells (*a*), cells treated with trypsin for 40 min (*b*), and cells allowed to recover after addition of trypsin inhibitor for 2.5 h with (*c*) and without (*d*) cycloheximide were collected, suspended at a density of 10^8 cells ml^{-1} in 5 ml aliquots of solution containing 20 mM K/Na_2 phosphate, pH 6.5, 50 mM NaCl, and 2% glycerol, and frozen at -20 °C. After thawing, the disrupted cells, now ghosts, were washed three times in phosphate buffer containing 1 mM $CaCl_2$ and 10 mM EDTA and resuspended therein at a density of about 10^7 ghosts ml^{-1}. Aliquots (1 ml) in tubes were incubated at 22 °C on a shaker. After 45 min, the population was examined microscopically to determine the distribution in groups of 1–3 (A), 4–5 (B), 6–10 (C), 11–15 (D), 16–50 (E), 51–100 or more (F).

Carbohydrate-mediated elimination of avian plasma glycoprotein in mammals

THE heavily glycosylated[1] plasma protein α_1-acid glycoprotein (α_1AGP) is known to occur in numerous mammalian species[2]. Its biological significance is still largely unknown[3] but together with fibrinogen and caeruloplasmin it belongs to a particular group of plasma proteins (acute phase reactants)[4], the synthesis of which is a response to trauma.

We isolated from chicken serum an α_1-acid glycoprotein which resisted precipitation at pH 3.0 and 2 °C with a combination of ammonium sulphate and trichloroacetic acid at the respective final concentrations of 2.18 and 0.075 M. Further purification of this protein was accomplished by ion exchange chromatography on Amberlite IRC-50 resin[5]. The product migrated with the mobility of human α_1AGP as a single band when electrophoresed in 7.5% polyacrylamide gel at pH 8.3. Its molecular weight, as determined by ultracentrifugation using a low speed equilibrium technique[6], was about 45,000 and it contained 44% (w/w) total carbohydrate (12.5% sialic acid[7], 16.0% hexosamine[8] and 16.0% neutral sugars[9]). Judged by these criteria this avian protein compares well with, and is therefore assumed analogous with, human α_1AGP.

Human α_1AGP and chicken α_1AGP were labelled[10] with different isotopes of iodine and injected simultaneously into adult chickens. The plasma protein-bound radioactivity curves closely paralleled each other, indicating no major difference in the catabolic rates of these proteins in the chicken (Fig. 1). By sharp contrast, when the same preparations were injected into New Zealand White rabbits the human protein underwent catabolism at rates which closely resembled rabbit α_1AGP,

Table 1 Means ± s. d. for the recovery of various iodine-labelled glycoproteins with the rat liver

Experiment		Marker protein		Test protein		Treatment*
Code	No.	Type	Dose in liver (%)	Type	Dose in liver (%)	
A_1	7	$H\alpha_1AGP$	12.7 ±4.7	$CH\alpha_1AGP$	57.6 ±8.7	None
A_2	3	—	—	$CH\alpha_1AGP$	61.4 ±4.3	None
A_3	3	—	—	$CH\alpha_1AGP$	60.4 ±1.5	None
B	3	$H\alpha_1AGP$	10.4 ±2.7	$CH\alpha_1AGP$	57.8 ±5.6	$R\alpha_1AGP$ (2.4 mg per 100 g)
C	4	$H\alpha_1AGP$	12.9 ±1.2	$CH\alpha_1AGP$	35.6 ±4.9	$H\alpha_1AGP$ (3.1 mg per 100 g)
D	4	$H\alpha_1AGP$	7.4 ±1.2	$CH\alpha_1AGP$	8.4 ±1.2	$HAs\alpha_1$ AGP (3.0 mg per 100 g)
E	5	HTr	10.2 ±0.5	HAsTr	42.3 ±1.2	None
F	5	HTr	9.0 ±1.1	HAsTr	11.7 ±1.3	$CH\alpha_1AGP$ (2.7 mg per 100 g)
G	4	$H\alpha_1AGP$	8.6 ±1.5	$HAs\alpha_1AGP$	98.1 ±0.8	None
H	5	$H\alpha_1AGP$	7.4 ±2.4	$HAs\alpha_1AGP$	79.5 ±5.5	$CH\alpha_1AGP$ (2.3 mg per 100 g)

H, human; CH, chicken; R, rat; Tr, transferrin; As, selectively desialylated with neuraminidase.
* Given intravenously 1 min before the dose.
Complete desialylation of human α_1AGP was achieved by incubating purified neuraminidase[8] (0.03 units) with 10 mg α_1AGP at pH 5.2 for 18 h at 37 °C. The enzyme was removed by passing the incubate through a column (10 cm × 1 cm) of Amberlite IRC-50 equilibrated with 0.015 M Na phosphate, pH 6.0. Under these conditions asialo-orosomucoid passed through the column and neuraminidase was adsorbed by the resin.
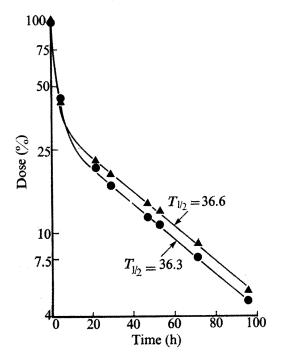The preparation of human asialotransferrin, free from neuraminidase activity, has been described elsewhere[16].

whereas more than 90% of the chicken α_1AGP was eliminated in the first 4 h of the experiment (Fig. 2).

To determine the mechanism responsible for the premature elimination of the avian α_1AGP by the rabbit, male Sprague–Dawley rats, of 297 ± 38 g mean body weight, were given an intravenous dose of an iodine-labelled test glycoprotein (10–20 µg per 100 g body weight) together with a differently labelled protein marker for trapped plasma volume, such as human transferrin (Behringwerke, Germany) or human α_1AGP (prepared by the same technique as the chicken α_1AGP). After an interval of 5–6 min, 2.8 ± 0.6 ml blood were withdrawn per 100 g body weight from the heart, under sodium pentobarbital anaesthesia. The liver was removed and immediately homogenised in 50–70 ml 0.89% NaCl containing 16% 2-octanol by volume. Duplicate aliquots of the homogenates were assayed for radioactivity in a Packard model 5986 multichannel analyser.

Experiments A_1, A_2 and A_3 (Table 1) demonstrate the respective behaviour of three chicken α_1AGP preparations in

Fig. 1 Protein-bound radioactivities in the plasma of a hen (1.8 kg) which received a mixture of chicken ^{125}I-α_1AGP (●) and human ^{131}I-α_1AGP (▲) intravenously.

the rat, each of which was obtained from the pooled sera of more than 125 chickens. A reproducibly large fraction of the dose was recovered with the liver after administering any of these preparations. Injection of an excess amount of rat α_1AGP beforehand had no effect on the recovery of this fraction (experiment B). Similar quantities of human α_1AGP, however, significantly ($P < 0.001$) reduced the accumulation of chicken α_1AGP in the rat liver (experiment C), and desialylated human α_1AGP abolished it altogether (experiment D). These observations strongly suggest that, when placed in a mammalian system, chicken α_1AGP competes for the same hepatic plasma membrane receptor, identified[12] as being responsible for the rapid elimination of mammalian asialoglycoproteins. This view
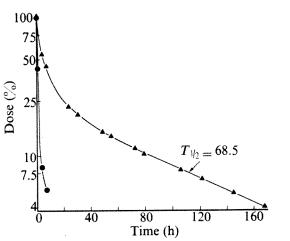

Fig. 2 Protein-bound radioactivities in the plasma of a rabbit (3.8 kg) following the intravenous injection of chicken ^{125}I-α_1AGP (●) and human ^{131}I-α_1AGP (▲).

is further strengthened by the marked interference with the hepatic clearance of human asialotransferrin and human asialo-α_1AGP by preinjected chicken α_1AGP. Thus, hepatic uptake of human asialotransferrin[13] was almost completely blocked by chicken α_1AGP (experiments E and F), whereas that of human asialo-orosomucoid was significantly ($P < 0.001$) reduced (experiments G and H).

Since the hepatic asialoglycoprotein receptor operates by recognising exposed galactose residues following the removal of sialic acid from glycoproteins[14], we determined whether native chicken α_1AGP contained such residues in a terminal position. The protein was treated with galactose oxidase and subsequently reduced by tritiated sodium borohydride[15]. By reference to the

radioactivity incorporated into a human asialotransferrin standard in these conditions, the quantity of terminal galactosyl groups in chicken α_1AGP was estimated to be 2.61 residues per molecule. The corresponding value for human α_1AGP was 0.97 residues which probably explains the partial blocking effect of this protein (experiment C).

The chemical and *in vivo* evidence presented suggests that chicken α_1AGP represents a naturally occurring glycoprotein which cannot survive in the mammalian circulation because a significant number of its carbohydrate chains terminate in galactose. Its affinity for the hepatic asialoglycoprotein receptor of rats is stronger than that of human asialotransferrin but weaker than that of human asialo-α_1AGP. The occurrence of such a glycoprotein in the avian circulation indicates that the chicken liver is either devoid of an asialoglycoprotein receptor altogether or that its requirement for a minimal number of exposed galactosyl groups is fundamentally different from the mammalian system[14].

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New theory for receptor mechanism of carotid body chemoreceptors

EVER since De Castro[1] first ascribed a chemosensory function to the carotid body (later confirmed by Heymans, *et al.*[2]), there has been much discussion and controversy over the precise mechanism whereby stimulation of the afferent fibres is achieved[3,4]. The controversy initially centred around the nature of the neurotransmitter released by the glomus cell, which was assumed to be highly sensitive to hypoxia and less sensitive to hypercapnia and acidaemia. Perhaps the most generally accepted theory was that the glomus cells respond to hypoxia by releasing a neurotransmitter that initiates an increase in firing rate of the nerve fibres terminating on the glomus cells. The afferent nature of these fibres was originally deduced by De Castro[5] from light microscopic examination of the nerve fibres innervating the carotid body. The early ultrastructural studies, however, failed to show clear evidence of afferent types of nerve ending in synaptic contact with the glomus cells. On the contrary, these nerve endings were purported to be pre-synaptic and therefore efferent in function because they contain
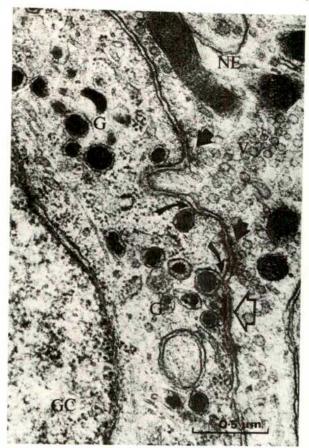

Fig. 1 Electron micrograph of the synaptic contact between a glomus cell (GC) and a nerve ending (NE) from the carotid body of the duck, *Anas platyrhynchos*. The glomus cell contains large numbers of electron-dense granules (G) which are thought to contain dopamine. In contrast, the nerve ending contains agranular vesicles (V) of the cholinergic type. Areas of synaptic contact between the glomus cell and nerve ending are interpreted as being efferent (solid arrows) and afferent (open arrow) on the basis of the location of the storage vesicles and associated membrane thickenings. Some electron-dense granules in the glomus cell seem to contact the cell membrane (curved arrows). Material prefixed with glutaraldehyde, postfixed with osmium tetroxide and embedded in Epon.

large numbers of agranular 'synaptic' vesicles[6-8]. This conclusion was further substantiated by Biscoe *et al.*[9], who showed that intracranial decentralisation of the IXth cranial nerve caused degeneration of nerve endings on glomus cells. These findings led to an alternative theory of chemoreceptor action[3]. In this case the chemosensory function is attributed to numerous free nerve endings of small diameter, which are found in the carotid body tissue. The glomus cells and associated nerves are held to form part of an efferent inhibitory feedback system, in which release of biogenic amines, that are known to be present in large quantities in glomus cells, depresses chemoreceptor activity.

Work, however, on cats[10] and on ducks (P. J. Butler and M. P. Osborne, unpublished) has shown, in agreement with De Castro[5], that decentralisation of the nerves leading to the carotid body does not result in degeneration of any of the nerve terminals in synaptic contact with the glomus cells. Furthermore, recent electron microscope studies have shown that both afferent and efferent synaptic complexes occur between the glomus cells and nerve endings[11]. Our own investigations on the duck have shown that both afferent and efferent synaptic contacts occur between the glomus cell and the same nerve ending (Figs 1 and 2). This latter observation thus supports the suggestion made by Hess and Zapata[10] that "the sensory terminals on the glomus cells might well have in addition to their afferent functions, efferent functional effects like the presynaptic dendrites of the brain, which also pre-
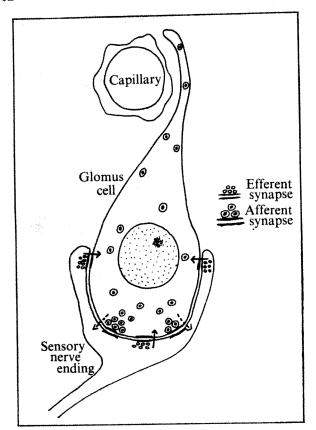
Fig. 2 Structure of a glomus cell and its associated nerve ending. The glomus cell has one or more elongated processes which come into close proximity with the capillaries. The nerve endings are usually cup-shaped (chalice-like) engulfing the basal region of the glomus cell. Afferent and efferent synaptic contacts are shown. The arrows show synaptic conduction during hypoxic conditions, that is, a reduction in neurotransmitter secretion at the afferent synapses (– – – →) and an increase in neurotransmitter release at the efferent synapses (——→).

choline (ACh) increases the rate of chemosensory afferent discharge. They therefore suggested that the glomus cells release ACh in response to natural stimuli, and that it is this chemical that excites the afferent fibres. There are unfortunately several strong arguments against this hypothesis, the most important of which are: the glomus cells contain large amounts of catecholamines, in particular dopamine[13], and this substance, as well as other catecholamines, has a marked depressant effect on spontaneous chemoreceptor discharge[14]; cholinergic blocking agents, although they abolish ACh sensitivity, do not abolish the response to natural stimuli[12,15]. Consequently if a neurotransmitter is released from the glomus cells there would seem to be little support at present for the proposal that ACh is the substance in question[4].

Taking all of the above evidence into account we have developed a new theory for carotid body chemoreceptive activity. The theory (Fig. 3) is that, in the absence of any physiological effect of the glomus cells, the sensory nerve fibres spontaneously discharge, that is, they are endogenously active[16]. This spontaneous activity could result from a constant sodium current flowing at the nerve terminals which could act like a generator potential and depolarise the spike-generating zone to initiate action potentials[17]. The frequency of discharge is controlled by secretion of an inhibitory transmitter, dopamine, from the glomus cells. Thus a high rate of dopamine secretion would hyperpolarise the nerve endings, possibly by increasing their potassium conductance[18], and reduce the discharge frequency. When blood gas tensions are normal the rate of dopamine secretion is high, thus attenuating the sensory fibre discharge frequency. In hypoxic conditions, the release of dopamine from the glomus cells is reduced, allowing the nerve endings to return to their depolarised state. Apart from increasing the afferent discharge frequency, depolarisation of the nerve endings in our scheme also causes release of a neurotransmitter, possibly ACh, from the efferent synapses of the nerve terminals. This efferent neurotransmitter acts on the glomus cells to reduce further their rate of dopamine secretion. This results in a larger depolarisation of the nerve terminals which instigates an even greater increase in discharge frequency. Thus in our model the efferent synaptic contacts act as a positive feedback loop. If ACh is the efferent neurotransmitter, then this could explain why cholinergic blocking agents suppress the afferent discharge, but do not abolish the response to hypoxia[12]. Also the positive feedback loop could account for the hyperbolic response curve of single-unit preparations to hypoxia[19]. We also believe that this model

sumably play a dual role". Present ultrastructural evidence therefore is strongly indicative of the glomus cell being part of an afferent system.

The assumption that the glomus cells are indeed the chemoreceptive cells is difficult to reconcile with neuropharmacological studies. Eyzaguirre and Zapata[12] have shown that acetyl-
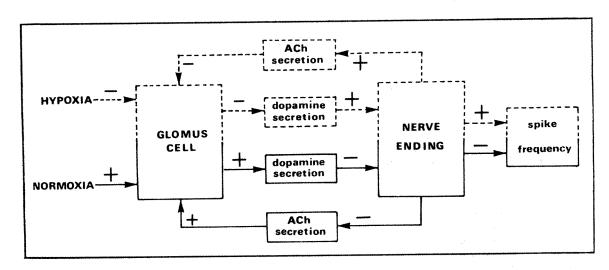

Fig. 3 Schematic flow diagram of a carotid body receptor during hypoxia (upper, dotted lines) and normoxia (lower, solid lines). During normoxia the glomus cell secretes dopamine which reduces afferent discharge frequency. In these conditions little or no ACh is released from the nerve endings at the efferent synapses. During hypoxia the glomus cell reduces its rate of dopamine secretion which leads to an increase in afferent discharge frequency. At the same time ACh is released from the efferent synapses and further suppresses dopamine release from the glomus cell, thus forming a positive feedback loop.

adequately explains the presence of ACh within the carotid body[12] and the presence of acetylcholinesterase[20] and dopamine[13] within the glomus cells It also accounts for the evidence that ACh[12] and dibenzyline[14] (an α adrenoceptor blocking agent) elevate afferent chemosensory activity and that catecholamines, notably dopamine, block the afferent discharge[14]

The novel feature of our theory is the proposal that in normoxic conditions, the glomus cells secrete an inhibitory transmitter, and in hypoxic conditions the rate of secretion of this transmitter is reduced Also, during hypoxia we envisage the nerve endings increasing the rate of release of their neurotransmitter substance These two opposite changes in metabolic rate need not be mutually exclusive if we suppose the low affinity cytochrome a_3 present in the carotid body[21] is located in the sensory (glomus) cells and that the normal cytochrome a_3 (that is, high affinity) is present in the nerves The rate of dopamine secretion from the glomus cells could be linked to a metabolic process(es) which is dependent on the oxygen tension Thus a change in rate of this process might act directly on the secretory mechanism or produce a change in membrane potential of the glomus cell Therefore it is possible that a depolarisation of the glomus cell would increase, and a hyperpolarisation decrease, the rate of secretion of dopamine Several attempts have been made to record changes in membrane potential of the glomus cells which are associated with natural or artificial stimuli In one study[22] the authors found no changes, but they could not be sure which type of cell they were recording from Other authors[23] reported that glomus cells were difficult to depolarise and that the sole potential change they recorded was a hyperpolarisation, and this was only in response to high levels of cyanide ions The response of the glomus cells, however, need not necessarily involve large changes in membrane potential Data from vertebrate photoreceptors[24] and lateral line organs[25] show that receptor potentials are of the order of microvolts rather than millivolts Since, in these sense organs, changes of a few microvolts are sufficient to alter the rate of neurotransmitter release, it seems that changes in membrane potential of similar magnitude could equally be effective in altering the rate of dopamine release from the glomus cells Similarly, the efferent neurotransmitter could reduce dopamine secretion from the glomus cells by hyperpolarising effects of less than one millivolt

Our theory does not exclude the possibility of an independent efferent nerve supply to the carotid body for which there is substantial neurophysiological[26-29] and some ultrastructural evidence[30] Whether this supply is to the vasculature[31], to the glomus cells[32], or to both is unresolved, but whatever the case, the presence of an efferent system need not invalidate our proposals

Bursts of antidromic impulses in the chemosensory fibres may be expected to increase afferent discharge in our model by the release of ACh This would occur if it is assumed that the antidromic spikes invade the nerve terminals to cause release of the efferent transmitter This assumption need not necessarily be correct It could equally be argued that antidromic spikes would "induce a build-up of positive afterpotentials in the region of orthodromic spike initiation, and produce an elevation of the threshold and depression of discharge"[33] Such a depression of afferent discharge is known to occur following antidromic stimulation in a number of sense organs including the carotid body[33]

Regeneration studies[34] indicate that a 12–18-month-old neuroma on the sinus nerve has similar physiological properties to the intact carotid body Unfortunately the authors do not mention whether they checked the denervated carotid bodies to see if reinnervation had occurred Until this possibility is eliminated we do not feel that these studies seriously challenge our theory of carotid body function A more detailed account of our ultrastructural studies will be published elsewhere

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Pentobarbital modulates transmitter effects on mouse spinal neurones grown in tissue culture

GENERAL anaesthetics depress postsynaptic excitatory transmission in the vertebrate central and peripheral nervous systems[1-4] although preserving or prolonging both presynaptic[5-6] and postsynaptic inhibition[7-10] The mechanisms underlying these cellular events and their precise relationship with the phenomenon of general anaesthesia in mammals have not been elucidated We have used various invertebrate preparations to show that pentobarbital and other general anaesthetics operate at a postsynaptic level to depress Na^+-dependent postsynaptic excitation without affecting either Cl^-- or K^+-dependent postsynaptic inhibition[11,12] Here, using intracellular recording from mouse spinal neurones grown in tissue culture, we show that pentobarbital depresses glutamate excitation and prolongs γ-aminobutyric acid (GABA) inhibition in most cells, through a postsynaptic mechanism

Spinal cord tissue dissected from 13-d mouse embryos was dissociated and plated at a density of 10^6 cells per dish (35 mm diameter) The culture medium consisted of modified Eagle's MEM plus animal serum (see ref 13 for details) Cultures were exposed briefly to a metabolic inhibitor (to suppress growth of background cells) and then allowed to mature for at least 3 weeks before use For experiments, culture dishes were placed in a chamber on the stage of an inverted, phasecontrast microscope Temperature and pH of the medium were controlled Standard electrophysiological techniques were used for intracellular recording and iontophoretic drug application A bridge circuit permitted current to be injected through the recording pipette so that input resistance could be measured $MgSO_4$ was added at the time of study to make a final con-

centration of 10 mM to eliminate spontaneous synaptic activity and suppress presynaptic transmitter release Neuronal responses to transmitters were not affected

The somata of large multipolar spinal cord cells were impaled under direct vision using electrodes filled with 4 M K⁺ acetate Two or more independent iontophoretic pipettes filled with various drugs (0 5 M glutamate, pH 8, 1 M GABA, pH 3 5, 0 5 M glycine, pH 3 5, 0 5 M Na⁺ pentobarbital, pH 9 2) were then positioned close to one another at the surface of the cell Drugs were iontophoresed, using the appropriate polarity current, to cell somata so that the recording pipette could detect maximally the effects of the drugs The results reported here were obtained from a population of neurones with resting potentials more negative than −45 mV and action potentials of 50 mV or more in amplitude Dorsal root ganglion cells were also present in the cultures but the results regarding these cells are still inconclusive

Glutamate, a putative transmitter mediating synaptic excitation[14], depolarised more than 95% of spinal cells examined (Fig 1) The dose-dependent, rapid depolarisation typically elicited spike discharge, was associated with a small decrease in membrane resistance and had an extrapolated inversion potential which was usually around −20 mV (ref 15 and N Brookes, unpublished)

Application of GABA or glycine, putative inhibitory transmitters in the central nervous system (CNS)[14], markedly reduced membrane resistance and depressed spike discharge in a dose-dependent, reversible manner in 85% and 50%, respectively, of cells examined (Fig 1b) The equilibrium potential for these responses was typically near the resting potential and in all instances where the effects of these two drugs could be compared on the same cell, they had identical equilibrium potentials[15] Pentobarbital also decreased the membrane resistance (Fig 1c) in a dose-dependent, reversible manner in about 80% of cells tested The decrease in resistance associated with pentobarbital amounted to 5–15% of the resting input resistance, although decreases of 25% were observed with very large applications The resistance change was often accompanied by a 1–3 mV shift in membrane potential, usually in the hyperpolarising direction The slowly developing pentobarbital effect required more iontophoretic current than the rapidly developing GABA or glycine responses (Fig 1c) Pentobarbital did not seem to affect the height of the spike, its duration or threshold

Focal applications of pentobarbital depressed the depolarising responses to glutamate (Fig 1a) in a dose-dependent, reversible manner (12 of 14 cells tested) without diminishing the membrane responses to either GABA (Fig 1b) or glycine The depressant effects of pentobarbital could be seen in the absence of any measurable effects on membrane properties, when low iontophoretic currents of the drug were used Direct effects of pentobarbital on membrane properties, when present, could account for only a small percentage of the depression of the glutamate response In Fig 1a, for example, a 55% depression of the glutamate response by pentobarbital was associated with a 13% decrease in membrane resistance, while in Fig 1b (panels on the right) a 12% decrease in membrane resistance was present during the 38% depression The decrease in membrane resistance induced by GABA or glycine was never antagonised by pentobarbital (Fig 1b and d) More than 50% of the cells studied showed a prolongation of the GABA response in the presence of pentobarbital (Fig 1b and d) This effect was analysed quantitatively by examining the change in membrane conductance following iontophoresis of GABA as a function of time Semi-log plots of these data revealed linear relationships from which half-times ($t_{1/2}$) for the restoration of the control conductance were obtained (our unpublished data) In Fig 1b, for example, the $t_{1/2}$ increased from 2 5 s for the response under drug-free conditions to 3 9 s for the response in the presence of pentobarbital Pentobarbital did not prolong the glycine response in four cells tested

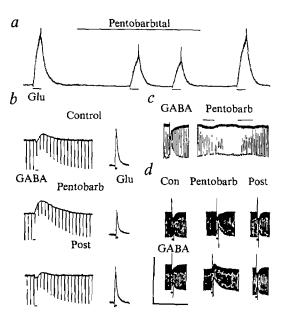Pentobarbital also tended to change the polarity of the GABA

Fig. 1 Effects of iontophoretically applied pentobarbital on nerve cells in tissue culture and its interactions with putative transmitters *a*, Pipettes containing pentobarbital and glutamate were positioned at the same site on the cell soma Standard pulses of glutamate (20 nA, 3 s) were applied alone (indicated by labelled bars or filled circles) and in the presence of pentobarbital (50 nA) Membrane potential was −58 mV Upward deflections are positive for all records *b*, Similar experiment using glutamate, GABA and pentobarbital pipettes near the cell soma Records show drug responses to constant pulses of the two transmitters (8 nA, 0 3 s for glutamate and 45 nA, 1 5 s for GABA) before (control), during (pentobarbital) and seconds after (post) application of pentobarbital (30 nA) Downward deflections in GABA traces here and in panels (c) and (d) are voltage responses to constant current pulses reflecting membrane resistance during drug responses $t_{1/2}$ for the relaxation of the conductance change after the termination of GABA application was 2 5 s in control and 3 9 s during pentobarbital application (see text) Peak resistance decrease during GABA response was 52% in control compared with 54% during pentobarbital Membrane potential was −50 mV Calibration 25 mV, 10 s *c*, Comparison of effects of GABA and various doses of pentobarbital applied to the same membrane site Onset of GABA response (45 nA and 2 s) was much faster than pentobarbital effect (45 nA first bar, 25 nA second bar) Membrane potential was −50 mV Calibration 25 mV, 50 s *d*, GABA and pentobarbital applied to same membrane site on cell soma Top three records show the action of a constant pulse of GABA (20 nA and 3 s) before (con), during (pentobarb), and after (post) the application of pentobarbital (12 nA) Bottom three records illustrate the effects of a 24-nA application of pentobarbital The membrane hyperpolarised by 3 mV during the larger dose of pentobarbital and the GABA response inverted (see text) $t_{1/2}$ of the relaxation of the GABA conductance change was 1 8 s for the con and 4 s for the pentobarb response Membrane potential was −56 mV Calibration 35 mV, 50 s

response in a depolarising direction (Fig 1b and d, bottom panels) This could not be explained by a change in the resting potential induced by pentobarbital and may have resulted from an effect of pentobarbital either on an ionic gradient on which the GABA response depends, or on the basic ionic mechanism induced by GABA Alternatively, the GABA response in some neurones may be normally biphasic, as suggested by the control record in Fig 1b The effect of pentobarbital may thus be to accentuate the later, depolarising component of this response

The results demonstrate three specific effects of pentobarbital on mammalian CNS neurones (1) a depression of postsynaptic glutamate excitation independent of direct effects on membrane potential or resistance, (2) a variably present prolongation of the conductance change induced by GABA (an effect not seen in invertebrates), and (3) a dose-dependent, slowly developing increase in membrane conductance (usually 5–15%) These effects of pentobarbital may explain the invariable depression

of CNS excitability during pentobarbital anaesthesia The results suggest that the depression of excitatory postsynaptic potentials in the CNS could be the result of the selective, post-synaptic effects of the drug without necessitating major effects on presynaptic release The observed prolongation of the GABA response may help to explain the prolongation of presynaptic and postsynaptic inhibition, both of which are thought to be mediated by GABA[6,10,14,16-18] Direct membrane effects of pentobarbital might also contribute to general anaesthesia by causing either a slight shunting of neuronal membranes, thereby reducing the size of excitatory synaptic potentials, or a slight hyperpolarisation, thus moving the membrane from its firing level

There are several ways, therefore, in which the cellular effects of barbiturates could lead to generalised depression of neuronal excitability and general anaesthesia (1) selective, postsynaptic depression of glutamate excitation, (2) prolongation of GABA-mediated postsynaptic inhibition, (3) prolongation of GABA-mediated presynaptic inhibition leading to partial deafferentation and (4) direct effects on CNS neurones to depress their excitability The relative importance of each of these in producing general anaesthesia, as well as the effects of pentobarbital on other aspects of synaptology studied under controlled conditions in tissue culture, remain to be investigated

Note added in proof Since this report was submitted we have found similar pharmacological effects of pentobarbital on transmitter responses using anaesthetic concentrations (0 1–0 2 mm) applied from large-bore pipettes

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Increase in binding capacity for triiodothyronine in tadpole tail nuclei during metamorphosis

REGRESSION of the tadpole tail is under direct control of the thyroid hormones and provides a system for study of their mechanism of action[1] In this system triiodothyronine (T_3) is two to five times as active as thyroxine (T_4) in causing tail regression[2,3] Studies of the binding of ^{125}I-T_3 and ^{125}I-T_4 after incubation of hormone with sliced tailfin tissues have revealed high-affinity, saturable binding sites in the cell nucleus[4]—a maximum of 1,500 and 800 sites per nucleus for T_3 and T_4, respectively (Similar results have been obtained with tadpole liver cells[5]) The dissociation constants for T_3 and T_4 were almost identical (10^{-10} M) These results suggest that in
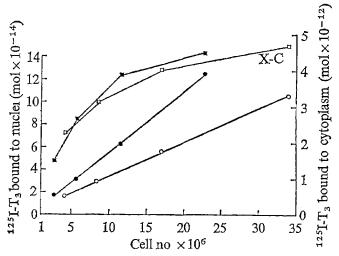

Fig 1 Dependence of binding of ^{125}I-T_3 to nuclei and to cytoplasm on the cell number Tailfin cell suspensions were prepared from stage X and XVIII–XIX tadpoles One ml of the cell suspension containing different amounts of cells was incubated with ^{125}I-T_3 (final concentration, 7 0 × 10^{-9} M) for 3 h at 25 °C ○, Binding to stage X nucleus, ●, stage XVIII–XIX nucleus, □, stage X cytoplasm, ■, stage XVIII–XIX cytoplasm Un-fractionated cell populations were used Contamination by blood cells was less than 3 % These cells appeared to be a mixture of epidermal, mesenchymal, and connective tissue cells[16] Separation of tailfin tissue cells from stage X and XX–XXI was performed on a Ficoll gradient (5–15 %, linear) Both stages had three visible band of cells and the relative widths of each band were almost identical for these two stages Therefore, it seems that there was no significant change in cell type of the tailfin in these preparations

amphibians the maximum number of binding sites rather than the affinity constant correlate with the biological activity of T_3 and T_4 In mammals, however, specific high-affinity binding sites for the thyroid hormones were found in nuclei[6-8] and correlate with the affinity constant

Derby[9] found that the sensitivity of tailfins to T_4 increases as metamorphosis progresses, but did not show whether this resulted from a change in the maximum binding capacity of the nucleus or to a change in the affinity constant for T_3 We have found that the maximum nuclear binding capacity for T_3 doubles during metamorphosis and that the affinity of the nucleus for the hormone decreases slightly during spontaneous metamorphosis

Rana catesbeiana tadpoles were classified according to stages as defined by Taylor and Kollros[10] Dorsal and ventral tailfins were sliced and digested twice in 5–10 volumes of amphibian Ringer phosphate buffer (ARB), pH 7 6, containing 0 10% glucose, 0 1% methylcellulose, 0 20% collagenase and 0 10% hyaluronidase (Worthington) for 30 min After filtration through nylon cloth (mesh size, 125 μm) the cell pellet was washed five times with ARB Cell yield from the sliced tissues was 50%, estimated from DNA recovery Cells were incubated with ^{125}I-T_3 (69 μCi μg^{-1}, Abbott) as described later

Table 1 Changes in maximum binding capacity and dissociation constant for T_3 by tailfin nuclei during metamorphosis

Tadpole stage	Binding sites per cell nucleus (±s e m)	Apparent dissociation constant (pM) (±s e m)
X	1,330±30	112±5
XV	1,580±40	105±4
XVIII–XIX	2,830±70	155±7
XX–XXI	2,410±30	169±4

Values were obtained from Fig 3, where the intercept of the ordinate represents the maximum number of binding sites and the slope represents the negative of the dissociation constant The data for stage XV were obtained by the same method as for the other stages 9 6 × 10^6 cells were incubated for 3 h at different concentrations of ^{125}I-T_3 The correlation coefficient for the linear regression was 0 97 (P < 0 01) While the correlation is high, it is not certain that the data fit only a single class of binding sites
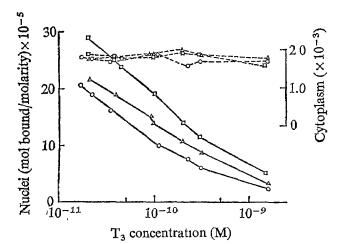
Preparation of tailfin cell nuclei was performed by the method of Samuels and Tsai[6] with 90% recovery of cell DNA[11] ^{125}I-T$_3$ analysed on a Sephadex G-25 column[4,5] contained 99 5% ^{125}I-T$_3$ and 0 5% ^{125}I-iodide The radioactivity of the nuclear pellet dissolved in 1 N NaOH and the extranuclear fraction was measured with a Beckman Model LS-250 liquid scintillation spectrometer with a counting efficiency of 48% using 0 5% Omnifluor, 1%, Cab-o-Sil, and 8% naphthalene in dioxane

The time course of binding of ^{125}I-T$_3$ to nuclei and cytoplasm was studied at 25 °C and 4 °C Both nuclear and extranuclear binding progressed linearly for 2 h and then plateaued Incubation was for 3 h in all subsequent experiments The extent of hormone binding was depressed 50% at 4 °C

The relationship between T$_3$ binding and the total number of cells used was studied at 7 0 × 10^{-9} M ^{125}I-T$_3$, using stage X and XVIII–XIX cells (Fig 1) A linear relationship was observed only in nuclear binding over the range tested From the intercept of the line, it was estimated that a cell nucleus from stage X contains 1,900 binding sites for T$_3$ and that of stage XVIII–XIX contains 3,300 sites, giving values slightly in excess of the values obtained by Scatchard plots (Table 1) At 7 0 × 10^{-9} M, the specific binding sites were saturated and contained some T$_3$, bound nonspecifically

Binding of ^{125}I-T$_3$ to nuclei and the extranuclear fraction was examined at different T$_3$ concentrations (Fig 2) using cells prepared from stages X, XV, XVIII–XIX, and XX–XXI The ratio of bound T$_3$ to the T$_3$ concentration of the medium is plotted against the concentration of T$_3$ in the medium at equilibrium The ratios for the extranuclear fraction remained constant and almost identical for different stages The ratios for the nucleus decreased as the hormone concentration increased, demonstrating that the nucleus contains high-affinity and saturable binding sites These data were replotted in a standard Scatchard plot (Fig 3)[12] Table 1 lists the maximum number of binding sites in the nucleus and their apparent dissociation constants as estimated from Fig 3 Maximum binding sites per nucleus remained almost unchanged through stage XV The present estimation of the maximum number of binding sites and their dissociation constants at stage X agrees with the results of binding experiments using tail slices[4] At stage XVIII–XIX, binding sites almost doubled compared with stage X and then decreased slightly at stage XX–XXI The affinity constant remained almost unchanged until stage XV and decreased

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Fig 3 A Scatchard plot of the binding of ^{125}I-T$_3$ by tail nuclei Binding of ^{125}I-T$_3$ to the nuclei was determined by incubating cell suspensions with radioactive hormone in 1 0 ml of ARB, containing 0 1% glucose and 0 1% methylcellulose After incubation, the cell pellet was obtained by centrifugation and the medium was saved to determine T$_3$ counts The cell pellet was washed by two successive suspensions and centrifugation in 2 ml of the cold incubation medium A nuclear pellet was prepared as described above Lines were obtained by linear regression with a correlation coefficient of 0 98–0 99 ($P < 0$ 01) Stage X, ●, stage XVIII–XIX, ■, stage XX–XXI, ▲

thereafter Since the available evidence suggests that the thyroid hormone level increases just before climax[13], it is probable that a higher fraction of the T$_3$ binding sites in the nucleus were occupied by endogenous T$_3$ at stages XVIII–XIX and XX–XXI than in early premetamorphic stages (X and XV)

The results presented here suggest that the maximum number of binding sites for T$_3$ in tail nuclei increased during spontaneous metamorphosis The increase in hormone sensitivity of tail tissue during metamorphosis may be the result of an increase in nuclear binding capacity for T$_3$ rather than an increase in the affinity constant, which seems to decrease as tadpoles metamorphose Tata reported that the acquisition of early metamorphic competence in whole *Xenopus* larvae is accompanied by the appearance of temperature-sensitive binding sites for thyroid hormones[14] The binding of very high concentrations of T$_4$ to temperature sensitive sites in tadpole liver nuclei was reported by Griswold *et al*[15] Samuels and Tsai[6] suggested that nuclear receptors for T$_3$ are not constant, but increase after exposure of intact cells to non-radioactive T$_3$ Therefore it is possible that the higher level of endogenous thyroid hormone at the onset of climax stages in the tadpole increases the nuclear receptors of the hormone, resulting in a greater tissue sensitivity to the hormone

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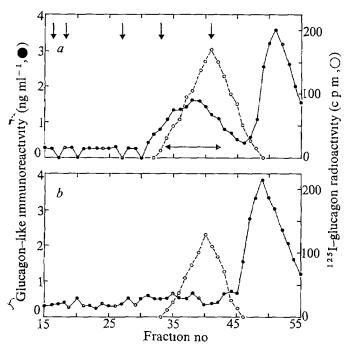
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Fig. 2 Binding of ^{125}I-T$_3$ to tail nuclei and cytoplasm at different concentrations of T$_3$ Tailfin cells were prepared from stage X, XVIII–XIX and XX–XXI tadpoles and were incubated for 3 h with different concentrations of ^{125}I-T$_3$ The number of cells used were 1 15 × 10^7 (stage X), 1 03 × 10^7 (XVIII–XIX) and 9 90 × 10^6 (XX–XXI) The incubation medium was saved for the determination of T$_3$ counts The ratio of bound ^{125}I-T$_3$ to the hormone concentration in the medium (M) was plotted against the medium hormone concentration at equilibrium Solid lines show the binding to nuclei Dotted lines show the binding to cytoplasm O, Stage X, □, stage XVIII–XIX, △, stage XX–XXI
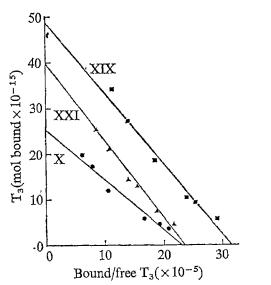
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Glucagon-like immunoreactivity in insect corpus cardiacum

SINCE Steele attributed hyperglycaemic activity to the corpus cardiacum of the cockroach neurosecretory system[1-3], it has been demonstrated in other species of Orthoptera[4-10] and in Diptera[11,12] and Hymenoptera[13] Although extracts of corpora cardiaca from the blowflies *Phormia*[11] and *Calliphora*[14] and the silk moth *Cecropia*[15] seem to be inactive, the hyperglycaemic hormone is liberated from the *Calliphora* gland by electrical[12] or mechanical[14] stimulation *in situ* Similarly, extracts from *Locusta*[6,16] and *Phormia*[11] are poorly active intraspecifically, but are hyperglycaemic when injected into the cockroach The nutritional state of the recipient is apparently crucial in determining the extent of the response[11]

In the best studied insects, the hyperglycaemic hormone functions similarly to glucagon in vertebrates the hormone increases carbohydrate levels in the circulating haemolymph

Fig. 1 Gel filtration of extracts of corpus cardiacum/corpus allatum complexes and brains from adult *M sexta* Tissue (21 complexes or brains) was suspended in 0 1 M Tris, 0 05 M NaCl, 0 25% bovine serum albumin, 1 mM diisopropylphosphorofluoridate (DFP), pH 7 6, lyophilised and extracted with 0 3 ml of 7 M guanidine hydrochloride containing a trace amount of ^{125}I-glucagon The extracts were separately applied to a column (0 9×42 cm) of BioGel P-10 equilibrated at 4 °C with Tris-albumin buffer containing pancreatic kallikrein inhibitor(300 U ml⁻¹) instead of DFP, and the components eluted at a flow rate of 5 ml h⁻¹ The 0 6 ml fractions were counted for radioactivity and 0 15 ml aliquots of each were removed for radioimmunoassay essentially as described[25] The antiglucagon serum was a gift from Dr L Heding, Novo Laboratories, Copenhagen Glucagon-like immunoreactivity (left axis, solid lines) and [12]I-glucagon radioactivity (right axis, dashed lines) were recorded for the complexes (*a*) and the brains (*b*) The elution positions of protein standards from the same column are indicated by the vertical arrows (*a*) which represent from left to right, albumin (void volume), soybean trypsin inhibitor (STI), cytochrome *c*, pancreatic trypsin inhibitor, and glucagon or ^{125}I-glucagon, respectively Fractions 34 to 42 indicated by the horizontal arrow (*a*) were pooled for further analysis
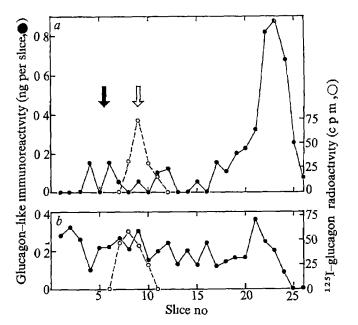

Fig 2 Polyacrylamide gel electrophoresis at pH 8 7 of extracts of corpus cardiacum and corpus allatum from *M sexta* Tissue (9 gland pairs) was extracted with 0 05 ml of 8 M urea containing trace amounts of ^{125}I-glucagon and bromphenol blue The extracts were applied to 10% polyacrylamide gels[28] containing 8 M urea (0 4×6 cm) and were subjected to electrophoresis at 22 °C Gels were sliced into 1 5-mm sections and placed in 0 5 ml of the buffer used for chromatography before counting for radioactivity After elution of the peptide components during 24 h at 4 °C, the supernatant fluid was withdrawn and subjected to immunoassay Glucagon-like immunoreactivity (left axis, solid lines) and ^{125}I-glucagon radioactivity (right axis, dashed lines) were recorded for the corpus cardiacum (*a*) and the corpus allatum (*b*) extracts Migration was from cathode (left) to anode The position of the tracking dye is coincident with the right axis The solid and open arrows (*a*) indicate the migration position of porcine glucagon and ^{125}I-glucagon respectively The additional negative charge in iodinated glucagon at pH 8 7 increases its anodal mobility relative to that of the unlabelled hormone Control experiments showed that the presence of urea or other gel components did not alter the sensitivity of the immunoassay

in vivo and stimulates glycogenolysis and the activation of phosphorylase in the fat body *in vitro*[5,6,17,18] By several criteria, the insect hormone also seems to be a low molecular weight polypeptide[2,6,8,10,13,16] We report here the identification of a highly acidic peptide with glucagon-like immunoreactivity from corpora cardiaca of the adult tobacco hornworm *Manduca sexta*

A survey for immunoreactive glucagon in tissues of *M sexta* showed that reactive peptides were present in corpus cardiacum/corpus allatum complexes and haemolymph, but not in brain, aorta, recurrent nerve or fat body The component from the complexes was eluted slightly ahead of [125] I-glucagon (molecular weight 3,500) during gel filtration on BioGel P-10 (Fig 1*a*) The elution volume of the immunoreactive component corresponded to that of a 4,500-dalton peptide As Fig 1*b* shows, gel filtration of an extract of brains from *M sexta* yielded no similar peak of glucagon-like immunoreactivity The peak of apparent reactivity at the far right of each profile in Fig 1 is caused by interference in the assay by guanidine hydrochloride and represents the inclusion volume of the column
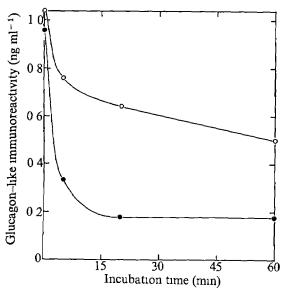
A closer examination of the distribution of the immunoreactive peptide showed that it was primarily associated with the corpus cardiacum rather than with the corpus allatum (Fig 2*a*, slices 21-25) Its rate of migration during electrophoresis at pH 8 7 is in agreement with its low molecular weight as determined by gel filtration, and further indicates a high acidity Nevertheless, the abilities of the serially diluted, pooled fractions 34-42 (Fig 1*a*) and porcine glucagon to compete with ^{125}I-glucagon for binding to the antibody

decreased in parallel Although the immunoreactivity profile of Fig 2b suggests that corpus cardiacum contains a single, major glucagon-like peptide, two or more rapidly migrating forms might not be separated under the conditions used here

Since mammalian glucagon[19] and the insect hyperglycaemic hormone[8,13] are sensitive to proteolytic degradation, we compared the susceptibilities of the immunoreactive component and porcine glucagon to trypsin (Fig 3) Although the immunoreactivities of both the insect peptide and glucagon are diminished by digestion with trypsin, the former is degraded either more slowly or to a lesser extent This result may be a consequence of the higher acidity of the insect peptide (Fig 2a) Quantitation of glucagon-like immunoreactivity after gel filtration or after polyacrylamide gel electrophoresis indicated that each pair of corpora cardiaca from *M sexta* contains 0 4–0 5 ng equivalent of glucagon This value should be regarded as a lower limit, however, since affinity of our antibody for the insect material may well be less than for porcine glucagon

Examination of sequence data shows that the structure of the vertebrate hyperglycaemic hormone glucagon has been well conserved during evolution the human,[20] bovine,[21] and porcine[19] hormones are identical, although the structures of the corresponding, slowly evolving cytochromes c differ by an average of 7% (ref 22) Similarly, avian glucagon differs from the human hormone by 7% (ref 23), whereas the difference between the corresponding cytochromes is about 12% (ref 22) Assuming from these limited data that the rate of mutational acceptance for glucagon is only about half that for cytochrome c or 1 5 accepted point mutations per 100 residues per 100 Myr,[24] we expect that a glucagon-like peptide might have changed by only 15% during the approximately 900 Myr since the divergence of insects and mammals[24] Neither this estimated extent of change nor the apparently higher molecular weight of the insect peptide would be likely to preclude reactivity with antibodies directed toward mammalian glucagon[25]

Weins and Gilbert first noted the nearly identical physiological

actions of the insect hyperglycaemic hormone and vertebrate glucagon[5] The tissue distribution of the glucagon-like immunoreactivity in *M sexta* parallels the distribution of the hyperglycaemic hormone in other insects[1,18,26,27] Furthermore, both immunoreactivity and hyperglycaemic activity are sensitive to tryptic degradation and behave similarly during gel filtration at neutral *p*H[13] The high acidity of the immunoreactive peptide is also consistent with the finding that hormone activity does not adsorb to cation-exchange resins[10,13] Although these chemical and physical similarities suggest that the insect hyperglycaemic hormone and the glucagon-like peptide are the same, final proof of their identity must await purification and sequence determination of the hyperglycaemic peptide

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Fig. 3 Effect of trypsin on the glucagon-like immunoreactivity in a gel-filtered extract of corpus cardiacum/corpus allatum complexes from *M sexta* Samples (0 4 ml) of the pooled fractions 34–42 from Fig 1 or of a solution of porcine glucagon (1 ng ml^{-1} in chromatography buffer) were incubated with diphenylcarbamyl chloride-treated trypsin (0 075 mg) at 25 °C for the indicated intervals The reaction was stopped by the addition of 0 1 ml of a solution of STI (8 mg ml^{-1}) All samples were subjected to the immunoassay and glucagon-like immunoreactivity was recorded for *M sexta* (○) and glucagon (●) The data points at zero time were obtained by adding STI to the appropriate solution before the addition of trypsin Control experiments showed that the presence of trypsin plus STI did not alter the sensitivity of the assay

Ethanol inhibition of transport of 5-hydroxyindoleacetic acid from cerebrospinal fluid

MUCH work has been directed at demonstrating the possible interaction of ethanol with levels of neurotransmitter amines or their metabolism in brain[1,2] We have previously demonstrated an increase in brain levels of 5-hydroxyindoleacetic acid (5-HIAA) in mice after both a single and chronic administration of ethanol[3] Our more recent work[4] has focused attention on the inhibition of transport of 5-HIAA from the central nervous system (CNS) by ethanol as the factor responsible for the increase in brain 5-HIAA after ethanol 5-HIAA diffuses from the CNS into cerebrospinal fluid (CSF), from which it is re-
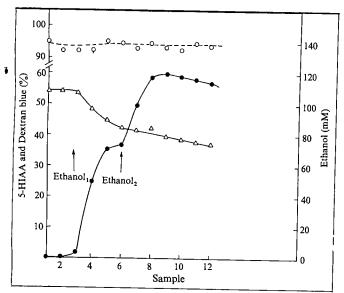
/ **Fig. 1** Transport of 5-HIAA from spinal subarachnoid space after ethanol administration The results illustrate one typical experiment of those treated statistically in Fig 2 A steady-state concentration of 5-HIAA and Dextran blue in outflow fluid during perfusion was established as follows Adult cats were anaesthetised with pentobarbital sodium (30 mg kg⁻¹ intraperitoneally) and prepared for perfusion as described previously[6] After the head of the animal was fixed in a stereotaxic instrument, an inflow cannula connected to a constant infusion pump was positioned in the cisterna manga Polyethylene tubing fixed in the lumbar subarachnoid space[6] served as outflow, its end was positioned 10 cm below the level of the cisterna magna and at the horizontal level of the lumbosacral cord Rectal temperature was recorded during the experiment and kept within normal range (± 0 5 °C) Artificial CSF (ref 7) containing 5-hydroxyindole-acetic-carboxyl-¹⁴C-acid (5-HIAA-¹⁴C, 400 ng ml⁻¹, specific activity 1 μCi 19 2 μg⁻¹) and Dextran blue (100 μg ml⁻¹) pH 7 4, was used for perfusion The rate of perfusion was 0 097 ml min⁻¹ With this perfusion rate approximately 120 min was required to get constant concentration of 5-HIAA-¹⁴C in the outflow fluid Thereafter samples of perfusate were collected at 20 min intervals To shorten the equilibration period the perfusion was started in a few experiments with a rate of 0 191 ml min⁻¹ and continued for 30 min, After this initial period, the perfusion rate was changed to 0 076 ml min⁻¹ and collection of samples was begun 60 min from the start of perfusion Ethanol concentrations in the perfusate were determined by gas chromatography[4] Radioactivity was determined by adding 0 1 ml of perfusate to 15 ml of Bray's solution[8] and subjecting the sample to scintilation counting Changes in concentration of Dextran blue were determined by measuring changes in absorbance at 630 nm from the initial value of 0 093 ± 0 002 which corresponded to 100 μg ml⁻¹ of Dextran blue Percentage transport for 5-HIAA (△) was calculated from

$$\left(1 - \frac{^{14}C \text{ in outflow}}{^{14}C \text{ in inflow}}\right) \times 100$$

Concentration of Dextran blue (○) in collection samples was expressed as percentage of the concentration being infused Ethanol (1 5 g kg⁻¹) was injected intraperitoneally twice with approximately 60 min between injections, and the concentrations of ethanol appearing in the perfusate (●) were determined

moved by a carrier-mediated transport into the bloodstream[5] The effect of ethanol on transport of 5-HIAA from CSF was studied by perfusing this acid through the spinal subarachnoid space from the cisterna magna to the lumbar region of cats This was shown to be a sensitive and reliable model for the study of transport of 5-HIAA from CSF (ref 6)

Figure 1 shows levels of 5-HIAA and Dextran blue in perfusate before and after two intraperitoneal injections of ethanol (1 5 g kg⁻¹) The concentration of Dextran blue did not change significantly during the experiment indicating that the bulk flow of the fluid was constant during the experiment On the other hand, a significant decrease in 5-HIAA transport was observed after ethanol administration (P < 0 001, analysis of variance for repeated measures[9])
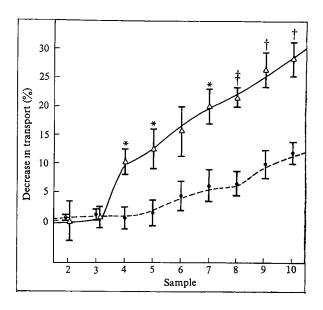
Figure 2 illustrates the mean decrease in 5-HIAA transport

after ethanol administration The administration of ethanol produced a 25–30% decrease in the transport of 5-HIAA during the course of our experiments Decreases in transport of 5-HIAA correlated well with the administration of ethanol (Fig 2) and the appearance of ethanol in the collection samples (Fig 1) The peak ethanol levels in CSF of cats after injection of ethanol (Fig 1) were similar to those previously noted in CNS of mice receiving 3 g kg⁻¹ ethanol[4] In some preliminary experiments (data not shown), ethanol (5 mg ml⁻¹ artificial CSF) was added to the perfusion fluid Such treatment produced no significant change in the transport of 5-HIAA compared with control cats Concentrations of ethanol in the perfusion outflow fluid, however, were found to be extremely low (that is, <1 mM), indicating that ethanol was quickly lost from the subarachnoid space Therefore, concentrations of ethanol necessary to inhibit 5-HIAA transport could not be maintained by this method

Our present experiments with cats injected with ethanol clearly indicate that ethanol inhibits transport of 5-HIAA from the CSF, and extend our earlier observations on the effects of ethanol on the transport of 5-HIAA from the CNS (ref 4) Previous work has demonstrated that measurable increases in the levels of 5-HIAA in brain do not occur simultaneously with peak ethanol levels in brain but approximately 60 min thereafter[3] Thus, it is interesting to note that in these experiments the inhibition of 5-HIAA transport by ethanol increases with time In addition, the partial inhibition of active transport systems, such as those for organic acids, which may be working at near maximal capacity[10,11], would result in a progressive accumulation of 5-HIAA with time Our studies with mice have also demonstrated that both the accumulation of 5-HIAA and the inhibition of transport systems for this organic acid by ethanol are reversible processes

Zarcone et al[12] demonstrated an increase in 5-HIAA in the lumbar CSF of alcoholics consuming ethanol A similar increase in 5-HIAA in cat cisternal CSF after injection of ethanol has also been observed (G E Goodman, M P Schulman, and M Radulovacki, personal communication) Our findings that ethanol inhibits transport of 5-HIAA out of CSF (Figs 1

Fig 2 Inhibition of 5-HIAA transport from spinal subarachnoid space by ethanol Perfusions of the spinal subarachnoid space were performed as described in the text Values represent mean ± s e of percentage change from steady-stage transport of 5-HIAA established initially Steady-state concentration of 5-HIAA was 50 9 ± 3 6% of infused 5-HIAA in control cats (●, n = 3) and 55 4 ± 2 4% in those cats which were to receive ethanol (△, n = 3) Time of the injection of ethanol as in Fig 1 Results were compared using Student's t test
*P < 0 1 †P < 0 05 ‡P < 0 02.

and 2) and CNS (refs 3 and 4) explain the underlying mechanism of these observations It is also interesting to note that inhibition of transport systems for certain organic acids not only inhibits the exit of acids from the CNS and CSF but also facilitates the penetration of such acids from blood into the CSF and CNS (ref 5) The increase in 5-HIAA or similar organic acids (biogenic acids) in the CNS may interfere with the normal metabolism of biogenic amines Biogenic acids have been shown to inhibit brain aldehyde reductases[13,14] which are responsible for the production of the alcohol derivatives of the biogenic amines (for example, MHPG) Biogenic acids, such as phenylacetic acid, have also been shown to inhibit certain decarboxylases[15,16] necessary for the biosynthesis of neurotransmitters such as catecholamines and γ-aminobutyric acid Further studies are in progress to elucidate the relationship between increases in CNS concentrations of organic acids and 'hangover' and withdrawal symptoms produced by ethanol

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Placental production and foetal utilisation of lactate and pyruvate

APPROXIMATELY 75% of foetal oxygen consumption of sheep can be accounted for by the uptake of glucose from the umbilical circulation[1-3] and catabolism of amino acids[4] The contribution to foetal energy metabolism of exogenous long-chain fatty acids[5] and fructose[1] seems to be negligible We report now that the placenta normally provides lactate for the foetus, in sufficient

quantities to account for 25% of foetal oxidative metabolism This finding invalidates the assumption that under normal physiological conditions there is a net flux of lactate from foetus to mother The assumption seemed reasonable in the past because the concentration of lactate in foetal blood is greater than in maternal blood and because of the formerly prominent hypothesis that anaerobic metabolism is very active in foetal life

Twelve Dorset and Western ewes at days 67–147 of gestation were starved for 48 h and then placed under pentobarbitol sedation (5 mg kg^{-1}) and spinal anaesthesia (6 mg pontocaine in hyperbaric glucose) Seven of these animals received polyvinyl catheters in the maternal femoral artery, the uterine vein, the foetal pedal artery and umbilical vein as described previously[2,6] In four cases the foetal vessels only were catheterised In one sheep, only the uterine circulation was studied

All lambs except two were live born spontaneously or by Caesarean section The two lambs found dead after spontaneous delivery were appropriate in size for their gestation ages of 95 and 115 d and had not been sampled for 25 and 7 d respectively before labour

After surgery all animals were placed in rectangular stalls and allowed food and water *ad libitum* Foetal and maternal catheters were flushed daily with heparin Procaine penicillin (600,000 U) and streptomycin (0 5 g) were administered on the day of surgery and the next three days Study began at least 24 h after surgery and extended to day 17 after surgery

Foetal (0 25 ml) and maternal (0 45 ml) blood samples were drawn simultaneously into 3-ml plastic syringes (Sherwood) surrounded by ice These were prepared on the day before sampling by inverting the syringe with needle attached into its prepackaged container The space between syringe and container was filled with water and stored at −4 °C to form an ice jacket around the syringe The blood samples were transferred immediately to preweighed plastic tubes (12 mm × 75 mm) containing 0 5 ml of 5 1% perchloric acid and agitated on a Vortex mixer (Scientific Industries) until precipitation was complete These tubes were then placed in a refrigerated centrifuge (International Equipment Co) and spun at 3,000 r p m for 20 min After separation of the supernatant, centrifugation was repeated before analysis Blood (0 2 ml) for analysis of oxygen content was drawn into heparinised glass capillary tubes (Scientific Products) in which 0 15 mg of sodium fluoride had been dried

Olsen's enzymatic method[7], utilising L-lactate dehydrogenase, was used to determine the concentrations of lactate and pyruvate Oxygen contents were determined with a Lex-O$_2$-Con (Lexington Instrument Corp) calibrated with distilled water saturated with oxygen at 0 °C All samples were analysed in triplicate

Lactate/oxygen quotients across the umbilical circulation were calculated on the basis of umbilical venous arterial differences (*v–a*) of lactate and oxygen, as follows

lactate/oxygen quotient = 3 (*v–a* lactate/*v–a* oxygen)

Expressed in this fashion, the lactate/oxygen quotient represents the fraction of foetal oxygen consumption required to metabolise

Table 1 Whole-blood lactate, pyruvate and oxygen contents (mM ± s e m)

	No of animals	Lactate Samples	mM	No of animals	Pyruvate Samples	mM	No of animals	Oxygen Samples	mM
Umbilical									
Artery (a)	11	40	2 05±0 15	6	23	0 25±0 08	6	23	3 31±0 17
Vein (v)			2 21±0 15			0 25±0 08			4 95±0 15
Uterine									
Artery (A)	8	42	0 86±0 07	6	24	0 13±0 02			
Vein (V)			0 93±0 07			0 14±0 01			
v–a			0 16*			0 00			1 64*
V–A			0 07*			0 01*			

*P < 0 001 (paired *t* test)

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Table 2 Lactate/oxygen quotients (L/O$_2$) in simultaneously drawn samples of umbilical venous (v) and arterial (a) blood

Animal	Age	v Lactate mM	v-a Lactate mM	v O$_2$ mM	v-a O$_2$ mM	L/O$_2$
J 019	96	1 78	0 40	5 98	1 52	0 79
	97	1 37	0 15	5 54	1 56	0 29
	98	1 52	—0 01	5 27	1 70	—0 02
	101	1 17	0 07	5 31	1 83	0 11
	103	2 29	0 15	5 22	2 55	0 17
	104	5 65	0 26	5 13	3 13	0 25
	108	3 69	0 08	3 05	1 83	0 13
032	114	1 49	0 09	5 45	1 92	0 14
031	125	2 15	0 11	3 71	1 56	0 22
	130	2 63	0 21	4 24	2 05	0 31
034	127	1 84	0 05	6 09	1 50	0 12
	128	1 86	0 09	4 96	1 13	0 24
	130	2 12	0 20	4 60	1 23	0 49
	131	1 82	0 04	4 78	1 38	0 09
	133	2 35	0 17	4 71	1 72	0 30
091	143	2 96	0 18	4 91	1 72	0 31
084	147	1 89	0 10	4 95	1 22	0 26
Mean		2 26	0 13	4 93	1 73	0 25*
±s e m		0 25	0 02	0 18	0 11	

*95% confidence limits according to Fieller's[13] theorem 0 16 to 0 32

the lactate acquired by the umbilical circulation to carbon dioxide and water

Table 1 shows that v-a lactate in the uterine and umbilical circulations was highly significant ($P < 0.001$) There was no significant veno-arterial difference of pyruvate concentration in the umbilical circulation There was, however, a relatively small, but statistically significant difference of pyruvate concentration between uterine veins and maternal arterial blood The lactate/oxygen quotient across the umbilical circulation was calculated on 17 samples from 6 animals (Table 2) The results indicate that lactate was being metabolised by the foetus in amounts that could account for 25% of the foetal oxygen uptake

Many studies performed in vitro have demonstrated that the placenta of several species has a relatively fast rate of aerobic glycolysis Murphey and Hawkins[8] demonstrated the phenomenon in the rat placenta, and Bell et al[9] found that it occurred only in the presence of chorionic epithelium Aerobic production of lactate by human placental tissue has been measured in vitro[10-12]

Our data demonstrate that lactate concentrations are greater in the umbilical and uterine veins than in their corresponding arteries In conjunction with the in vitro studies, these data indicate that the lactate acquired by the foetus from the umbilical circulation is produced by the placenta

This is the first demonstration that lactate produced by the placenta is an important substrate of foetal metabolism As demonstrated by the measurement of lactate/oxygen quotient, catabolism of the lactate acquired from the umbilical circulation to carbon dioxide and water would require one-quarter of the total foetal oxygen uptake This is equal to the estimated amount of oxygen needed for the foetal breakdown of amino acids[4] and half the oxygen required for the aerobic metabolism of the glucose which is delivered normally by the placenta to the sheep foetus[1-3]

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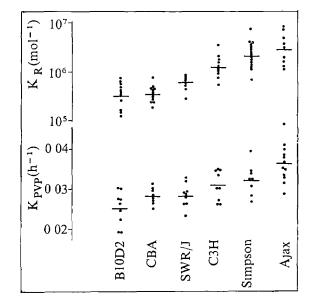1 Tsoulos, M G , Colwill, J R , Battaglia, F C , Makowski, E L , and Meschia, G , Am J Physiol , 221, 234–237 (1971)
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Relationship between macrophage clearance of PVP and affinity of anti-protein antibody response in inbred mouse strains

MACROPHAGES are important in immunity on their own, in delayed-type hypersensitivity, in phagocytosis of immune complexes, and in the afferent limb of antibody responses Evidence for the latter includes the increased immunogenicity of macrophage-processed antigen[1], and the requirement for macrophages in antibody responses involving cooperation between T and B lymphocytes[2] In addition, antibody titres can be increased or decreased by agents, such as adjuvants[3] or colloidal carbon[4], which probably act on macrophages Changes in either quantity or quality (affinity) of antibody, or both, may occur carbon[5] reduces the affinity of mouse anti-protein antibody but does not affect quantity, whereas adjuvant[6] increases both Differences in macrophage activity could therefore be responsible for differences in antibody affinity between mouse strains[7] Carbon clearance differs between strains[5,8], and has been found to be loosely related to affinity[5] We have used a new test of macrophages which shows differences of clearance function which relate closely to differences of antibody affinity

Polyvinyl pyrrolidone (PVP) clearance[9] was measured in normal 10 to 14-week-old mice of six inbred strains (B10D2(new), SWR/J, CBA, Simpson and Ajax) Results are expressed as the rate constant (K_{PVP}, h^{-1}) of the exponential fall in blood radioactivity between 18 and 48 h after intravenous injection of ^{125}I-PVP (Radiochemical Centre, Amersham) Mice of four of these strains were immunised with two weekly injections of 1 μg PVP (molecular weight, 360,000), and clearance of ^{125}I-PVP was tested one week later Affinity (K_R, mol^{-1}) and quantity (Ab_t, μM antibody binding sites) of antibody against human

Fig. 1 PVP clearance (K_{PVP}) in mice of six inbred strains, compared with affinity (K_R) of antibody for HSA (Data for K_R kindly provided by Dr M W Steward[7])
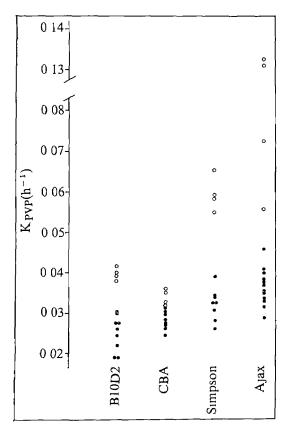
serum albumin (HSA) was determined in normal Ajax mice after saline immunisation[10], and in mice given 10 mg PVP (molecular weight, 360,000) intraperitoneally 24 h before each HSA injection

K_{PVP} differs significantly between the six strains of mice (Fig 1, $F=10\,4$, $P < 0\,01$, one-way analysis of variance), possibly on a genetic basis Variation within each strain exceeds the methodological variation[9], suggesting that environmental factors also contribute The correlation between the strain ranking order for K_{PVP} and antibody affinity (K_R) is striking ($r_s=0\,99$, $P<0\,01$, rank correlation on mean values), but there is no significant correlation between K_{PVP} and Ab_t ($r_s=0\,30$, $P>0\,05$) for the five strains for which values of Ab_t are available[7]

Tests of macrophage function have included *in vivo* clearance measurements using a variety of particles and *in vitro* tests of isolated monocytes or peritoneal cells[11] The different clearance tests may measure, to some extent, different things[8], and their relationship to *in vitro* function has been little studied All other clearance measurements give much faster clearance rates than ours, and use doses large enough to saturate the phagocytic capacity of the Kupffer cells[12] Such doses are likely both to stimulate and to block the function measured, and the magnitude of such effects differs between strains[8] The amount of PVP used is much smaller and does not cause dose-related blockade[9], so that PVP clearance may parallel the handling by macrophages of small quantities of antigen more accurately than do other tests Evidence that it does indeed measure a macrophage function includes the facts that the PVP is detected in macrophages and clearance and the hepatic uptake is reduced by carbon and by corticosteroids, but increased by oestrogens There is no evidence of antibody response at the time of testing, and repeat tests usually give similar values Large doses of unlabelled PVP blockade clearance of both PVP and carbon

The close relationship between PVP clearance and affinity of antibody to HSA suggests that the former measures a biologically important function, and supports the theory that variation

Fig. 2 PVP clearance (K_{PVP}) in normal mice (●) and mice immunised with 1μg PVP (○)

in macrophage function underlies differences in affinity of antibody response, as does the alteration of both antibody affinity and macrophage activity with carbon, adjuvant or oestrogen[5,6] Cells responsible for phagocytosis of PVP are involved in determining antibody affinity, since Ajax mice given 10 mg PVP before each injection of HSA showed significant ($P<0\,025$, Mann–Whitney U test) reduction in K_R (log mean $0\,12\times10^6$ mol^{-1}, compared with control mean of $0\,95\times10^6$ mol^{-1}), but Ab_t was unchanged (mean $2\,0$ μM, control mean $1\,8$ μM, $P>0\,05$)

Poor macrophage function probably leads to a low affinity antibody response because of poor selection of B lymphocytes The optimum response after adjuvantised immunisation[8] makes it unlikely that the limiting factor is a deficiency of suitable lymphocytes Perhaps PVP clearance correlates better with antibody affinity than does carbon clearance because PVP is more similar in size to the immunogenic microaggregates of soluble proteins than are carbon particles, and because the large dose of carbon partially blockades the macrophages[12] Our results do not imply that endocytosis of antigen is necessary for optimal antibody response[13] PVP clearance and antigen handling may both depend on overall macrophage activity and motility, or macrophage phagocytosis of PVP may depend on cell surface characteristics which are also important in cell interactions We do not know how the differences of antibody response we describe are related to those of mice selected for high and low titres of agglutinating antibody[14] These differences may also depend on macrophage function, as peritoneal cells from animals with low response show increased rates of antigen degradation[15] If they are related, the ability of the macrophage surface to bind and retain intact antigen molecules may be the critical function which determines the nature of the antibody response

Immune PVP clearance also differs between strains K_{PVP} was increased after immunisation (Fig 2) in all four strains tested, but more markedly so in the strains with high PVP clearance (Simpson and Ajax) than in the other two Immunised B10D2(new) and CBA mice clear PVP less well than many unimmunised Ajax mice There may be both low affinity antibody and defective clearance of immune complexes, and possibly also less antibody, but we do not know if there are differences in affinity of antibodies to PVP (T-cell independent and mainly IgM, ref 11) similar to those of IgG antibodies to cooperation-dependent protein antigens This individual variation of function may contribute to susceptibility to immunopathological processes such as immune complex disease[6], including that in man, in whom similar variation occurs (A G M and J F S, unpublished)

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Tumour-associated transplantation antigens of chemically induced sarcomata cross reacting with allogeneic histocompatibility antigens

INDIVIDUAL tumour-associated transplantation antigens (TATA) of chemically induced tumours and histocompatibility antigens (HA) share the ability of inducing transplantation immunity, and have other properties in common, such as the high degree of polymorphism[1-3] and a similar cell surface behaviour[4] A reciprocal relationship between the quantitative expression of TATA and H-2 antigens has been observed[5-6], suggesting the existence of common mechanisms either in their synthesis or in the control of their phenotypic expression Such indirect similarity prompted us to study the existence of cross reactions between TATA and HA We therefore determined whether 3-methylcholanthrene (MCA)-induced sarcomata possess TATA-containing allogeneic histocompatibility determinants Here we report data from two BALB/c fibrosarcomas, ST-5 and B-2

We investigated whether protection against the syngeneic tumours could be induced by immunisation with normal allogeneic tissues Groups of BALB/c mice were transplanted with skin or with kidney plus liver from syngeneic, C57BL/6J or C3Hf, mice, with skin of W/Fu rat, or were injected intraperitoneally with sheep red blood cells (SRBC) Ten days later all mice received 400 rad (whole-body) and were challenged on the following day with sarcoma ST-5 cells As shown in Fig 1a a significant protection against sarcoma ST-5 (as assessed by χ^2 test) was elicited by C57BL/6J skin or liver plus kidney grafts when compared with syngeneic skin graft ($P < 0.01$ at day 12 and 16, and $P < 0.05$ at day 20 after challenge), C3Hf skin graft was highly effective ($P < 0.001$ from day 8 to 32, and $P < 0.05$ at day 36) and a still stronger protection was achieved by C3Hf liver plus kidney graft ($P < 0.01$ from day 8 to 36), whereas no resistance could be induced by rat skin graft or by injection of SRBC No effect was induced by C57BL/6J or C3Hf liver plus kidney graft against the challenge of sarcoma B-2 (Fig 1b)

It seems, therefore, that TATA from sarcoma ST-5 cross reacted with HA of C57BL/6J and C3Hf strains It is unlikely that a nonspecific increase of the immune response caused by antigenic stimulation was responsible for the anti-tumour resistance induced by allografts, since rat xenograft and SRBC injection had no effect, and since sublethal X-irradiation strongly impairs primary immune response Moreover, no resistance could be induced with the same schedule against sarcoma B-2, which lacks TATA

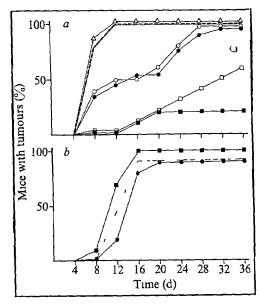These results were partially confirmed by serological studies

Fig 1 Antitumour resistance induced by allograft Groups of 10 BALB/c males 2 months old, received either a full thickness skin graft (15×20 mm), or a subcutaneous injection in the back of liver plus kidney fragments in 0 5 ml MEM+antibiotics, or were injected intraperitoneally with 10⁹ SRBC in 0 5 ml saline Liver plus kidney fragments were prepared by teasing and mincing the organs and the equivalent of one-third of the pool of one liver and two kidneys was given to each mouse Challenge 5×10⁴ ST-5 cells (a) or 2×10³ B-2 cells (b) in 0 2 ml MEM, subcutaneously in the flank All mice were examined for palpable tumours every 4 d after challenge Skin graft □, C3Hf, ○, C57BL/6J, ———, BALB/c, - - - -, W/Fa rat Liver plus kidney graft ■, C3Hf, ●, C57BL/6J, - - -, BALB/c, △, SRBC Sarcoma ST-5 originated from BALB/c fibroblasts exposed to MCA in a diffusion chamber placed intraperitoneally in a BALB/c mouse The treated fibroblasts, for experimental reasons not relevant to this study[7], were transferred to a (C3Hf×BALB/c)F₁ hybrid until they developed in a palpable nodule The BALB/c genotype of the tumour was assessed by retransplanting the nodule in BALB/c mice where it took promptly Sarcoma B-2 was obtained by syngeneic subcutaneous transplantation of in vitro spontaneously transformed BALB/c fibroblasts Both tumours were serially transplanted in unconditioned BALB/c mice, and used between the 8th and 15th in vivo passage At the time of the present experiments ST-5 was found to be immunogenic using the in vivo growth-excision assay[7], whereas B-2 showed no immunogenicity, no cross reactivity was found between the two sarcomas The individuality of the ST-5 TATA was also proved by the absence of cross reaction with other MCA-induced BALB/c sarcomas

Absorption with ST-5 cells significantly removed the cytotoxic activity of a BALB/c anti-C57BL/6J serum compared with absorption with BALB/c lymphocytes or with sarcoma B-2 ($P < 0.001$ at 15×10⁶ absorbing cell dose, Fig 2) In contrast, several attempts to absorb the cytotoxic activity of a BALB/c anti-C3Hf serum with either tumour failed

To demonstrate further transplantation antigens belonging to C57BL/6J and C3Hf strains on ST-5 cells, we compared the immunogenic strength of TATA of sarcoma ST-5 in the syngeneic against (BALB/c×C57BL/6J)F₁ (CB6F₁) and (C3Hf×BALB/c)F₁ (C3CF₁) hybrid mice We anticipated that if C57BL/6J or C3Hf determinants were part of TATA of sarcoma ST-5, the immunogenicity of this tumour in the hybrid mice should have been absent or lower than in BALB/c mice Growth-excision of ST-5 in CB6F₁ mice elicited no immunity against a subsequent challenge of the related cells, whereas only a slight reduction in protection was observed in C3CF₁ compared with BALB/c mice (Table 1)

TATA of sarcoma ST-5 seems to be complex Whereas anti-tumour resistance induced by C57BL/6J and C3Hf allografts may be explained as TATA possessing a unique determinant shared by both C57BL/6J and C3Hf HA and absent in BALB/c tissues, such as specificity 5 of k and b H-2 genotypes, or H-4ª antigens, in the hybrid experiment a clear

Table 1 Immunogenicity test of ST-5 sarcoma in syngeneic against hybrid mice

Experimental groups*	No of mice with tumour†		
	No of mice injected		
	BALB/c	C3CF₁	CB6F₁
Immune	5/16 (31)	14/27 (51)	12/26 (46)
Controls	14/20‡ (70)	23/28§ (82)	13/23¶ (56)
Protection achieved by immunisation	39%	31%	10%

*Antitumour immunity was obtained by growing a tumour fragment subcutaneously (controls receiving normal BALB/c tissues subcutaneously) When approximately 10 mm in diameter, tumours were surgically removed and controls sham-operated Eight days later all the animals were challenged subcutaneously with 10⁵ ST-5 cells prepared by trypsinisation

†Final check for tumour takes at 32 d after challenge Percentage of takes in brackets Difference in takes between control and immune mice of the same strain as evaluated by χ^2 test
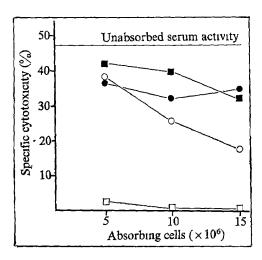
‡$P < 0.05$

§$P < 0.02$

¶Not significant

Fig. 2 Absorption of BALB/c anti-C57BL/6J serum by ST-5 and B-2 cells Serum obtained by immunising BALB/c mice with 4 weekly injections of C57BL/6J lymphoid cells The animals were bled 7 d after the last injection The ^{51}Cr-release test on C57BL/6J radiolabelled lymph node target cells[14] was performed at the serum endpoint dilution of 1 32 in the presence of guinea pig complement (B D Merieux, Marseille, France) The specific ^{51}Cr release was calculated as follows $100 \times$ experimental release-background/maximum releasable ^{51}Cr-background (where background represents ^{51}Cr release in the presence of heat inactivated complement) Tumour cells prepared by trypsin treatment for 20 min at room temperature and suspended in MEM, were incubated for 2 h at 37 °C before absorption Lymph node cells were obtained by mincing and teasing subcutaneous and mesenteric lymph nodes, and resuspended in MEM Absorptions were carried out by incubating 0 12 ml serum with 5×10^6 packed cells for 30 min at 37 °C followed by 30 min at 4 °C, in 2 ml test tubes To avoid excess dilution by a large cell pellet the absorption doses of 10 and 15×10^6 cells was reached by two and three repeated absorptions on 5×10^6 cells, respectively No gross difference was observed between ST-5 and B-2 cell pellet volume Each point is mean of three replicates ○, ST-5 cells, ●, B-2 cells, ■, BALB/c lymph node cells, □, C57BL/6J lymph node cells

reduction in immunogenicity was observed in CB6F$_1$ mice only This suggests that TATA of sarcoma ST-5 contains at least two components, one limited to C57BL/6J strain, tolerated in CB6F$_1$ and recognised as foreign in C3CF$_1$ hybrids, the other shared by both C57BL/6$_1$ and C3H$_1$ strains and therefore tolerated in both CB6F$_1$ and C3CF$_1$ hybrids A prevalence of C57BL/6J in the make-up of ST-5 TATA may also explain the failure of ST-5 cells to absorb the activity of a BALB/c anti-C3Hf serum Note, however, that C3Hf grafts induced a stronger antitumour resistance than the C57BL/6J ones

The possibility that a somatic hybridisation spontaneously occurred in the early passage of the tumour in the C3CF$_1$ host, may be responsible for the observed cross reactions, is unlikely, since the phenomenon has been reported to be infrequent[8], and since the subsequent serial passages in unconditioned BALB/c mice should have selected against hybridised cells In addition, a hybridisation between BALB/c and C3CF$_1$ cells could not explain the presence, on ST-5 cells, of C57BL/6J antigens not shared with C3Hf strain

We consider the origin of TATA of sarcoma ST-5 to be the result of carcinogen-induced random mutations at the level of histocompatibility genes, either of the H-2 system or of the minor loci Such a possibility is supported by the genetic linkage which seems to occur between TATA and the H-2 system[9]

Moreover, assuming that HA have a key function in the economy of cell life[10], only TATA identical or very similar to HA of normal cells should be selected for among the different neoantigens which appear at the initial stages of carcinogenesis[11] Alternatively, the possibility of a carcinogen-induced activation of silent histocompatibility genes normally expressed in allogeneic strains should be considered, as suggested for other systems[12,13] In this case one would expect

to find a perfect identity between TATA and a given foreig histocompatibility antigen

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Antibody to a molecularly-defined antigen confined to a tumour cell surface

TUMOUR-SPECIFIC antigens have usually been demonstrated with little knowledge of their molecular nature, for example by the residual reactivity with tumour shown by an anti-tumour serum which has been absorbed with normal tissue Certain tumours of B lymphoid cells which secrete immunoglobulin (Ig) provide a promising situation for identification of the antigen in molecular terms[1-3] Amino acid sequences in the variable (V) regions of Ig molecules determine not only antibody activity, but also the 'idiotypic' antigenic determinants of the molecule (for review see ref 4) In a normal B lymphoid clone the V regions on the surface Ig of the early members are the same as those on the Ig exported by specialised protein-secreting descendants and are essentially unique to this clone[5,6] The morphology and mode of Ig secretion in neoplastic cells are frozen at some stage in the range shown by their normally differentiating counterparts (Fig 1) Tumours with cells having both surface and export Ig have provided Ig in the host serum in amounts suitable for the conventional raising of anti-V sera These sera, by reacting specifically with the surface Ig of the corresponding tumour, demonstrated that the V regions can represent tumour-specific antigens[2,3] Furthermore, Lynch *et al*[1] demonstrated that mice immunised so as to produce antibodies to the V regions of such a tumour (a syngeneic mouse myeloma) were thereby afforded a measure of specific protection against tumour challenge

It is unlikely that anti-V sera raised in this way could be used therapeutically against the tumours concerned, because the exported Ig would represent a large extracellular barrier against access to the tumour cells Consider, however, the therapeutically interesting case of neoplastic B lymphocytes having only surface Ig (Fig 1) Are there definable idiotypic determinants on this surface Ig and is it possible routinely to raise the anti-V sera? Here we show that this is so in both cases for a guinea pig leukaemia

The L$_2$C leukaemia of strain 2 guinea pigs is a transplantable tumour, maintained by repeated passage *in vivo*, composed of large B lymphocytes[8] with scanty cytoplasm and no significant endoplasmic reticulum They bear IgM on their surfaces, approximately 100,000 molecules per cell being shed with a half life of about 5 h (ref 9) There is no evidence of IgM being delivered to extracellular fluid by any mechanism other than turnover on the cell surface[9]

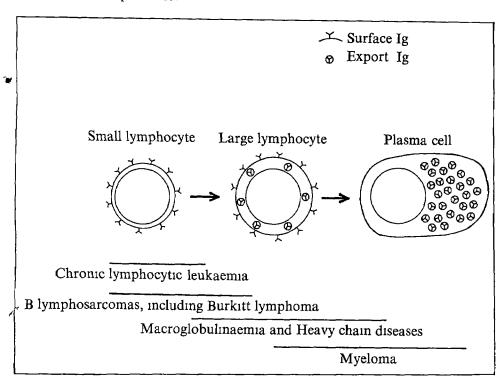Blood was collected by exsanguination of animals near

Fig. 1 Three morphological cell types distinguishable in the continuum of a normal maturing B lymphoid clone, with the associated modes of Ig secretion Note that some cells exhibit both surface and export Ig (ref 7) Neoplasms can seemingly arise at various stages to give clones in which differentiation is frozen The horizontal lines depict the ranges of cell types commonly seen among the indicated neoplasms The scheme is drawn for human neoplasms but is probably applicable broadly to all mammals

death as a result of leukaemia, and the leukaemic lymphocytes $(2 \times 10^5 - 3 \times 10^5 \, \mu l^{-1})$ were separated and washed thoroughly Limited proteolysis of the cells with papain[9,10] (0 06 mg ml^{-1} acting on 10^8 cells ml^{-1} for 30 min at 37 °C) cleaved the surface IgM *in situ* and released Fabμ fragments, bearing the V regions, into the supernatant From here the Fabμ was isolated by binding to a microcrystalline cellulose immunosorbent, with an approximate yield of 75 μg per 10^{10} cells[9]

Antibody to the V regions was raised by injecting the washed, Fabμ-laden immunosorbent from 3×10^{10} cells directly into two sheep (ref 11 and Fig 2) It should be noted that the schedule exposes the animals to particles on which the only foreign molecules are the cellulose matrix and the guinea pig Fabμ The Fabμ contains V and constant (C) regions, so passive antibody to the C regions was injected simultaneously with the antigen to promote the anti-V response and depress the anti-C[11-13]

Serum was obtained from the sheep 1-3 weeks after the second injection of antigen It contained low levels of two unwanted antibody activities to the Fabμ C regions, judged by precipitin reaction, and to guinea pig normal cell surfaces

judged by immunofluorescent staining of normal leukocytes These activities were removed by passaging IgG from the sera through normal strain 2 guinea pigs IgG from sheep normal serum was passaged similarly to provide a control The entire schedule (summarised in Fig 2), provided us with guinea pig serum containing sheep IgG, antibody or control, at approximately 2 mg ml^{-1} The 'antibody serum' and 'control serum' were tested for reactivity against L_2C cells and syngeneic normal lymphocytes (from lymph nodes, spleen and thymus) by immunofluorescence and inhibition of migration

Immunofluorescence was by the indirect technique with all manipulations carried out at 4 °C to prevent capping[14] Cells at 2×10^7 ml^{-1} in Eagle's MEM were mixed with an equal volume of antibody or control serum and incubated for 30 min They were then washed, treated with indicator reagent (fluorescein-conjugated IgG from rabbit antiserum to sheep IgG), washed again and examined in suspension The only positive reaction observed was with antibody serum and L_2C cells, which showed uniform circumferential staining with superimposed bright spots

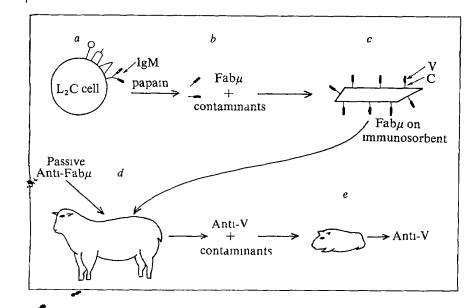Inhibition of migration was carried out as described by

Fig 2 Preparation of antibody to idiotypic determinants on V regions of surface Ig *a*, L_2C cells contain many surface antigens among which the Ig is a trace constituent *b*, Before exposure to the definitive immunosorbent the cellular supernatant undergoes a thorough preliminary absorption with a blank immunosorbent consisting of sheep normal IgG coupled to microcrystalline cellulose, thereby removing material adhering nonspecifically to the cellulose matrix *c*, The definitive immunosorbent was prepared by coupling purified sheep anti-guinea pig Fab'γ to the cellulose[11] *d*, Fabμ-laden immunosorbent was mixed with Freund's complete adjuvant and injected, in two doses five weeks apart, at multiple subcutaneous sites Each dose was accompanied by intravenous administration of 30 ml sheep antiserum to guinea pig serum Fabμ, in effect an antiserum to the C regions of Fabμ *e*, 100 mg IgG from unabsorbed sheep antiserum per 300-g guinea pig The animals were exsanguinated 6 h later, each yielding approximately 20 mg sheep IgG in 10 ml serum
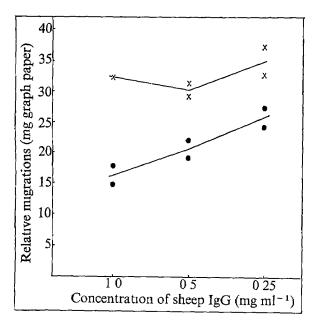
Fig. 3 Migrations of L_2C cells into nutrient medium containing sheep IgG passaged through normal guinea pigs ×, Normal IgG, ●, IgG from anti-V serum Note that a concentration of sheep IgG of 1 mg ml^{-1} is equivalent to approximately a 1 in 20 dilution of a sheep serum Migrations by normal (nodal and splenic) lymphocytes in the presence of normal or anti-V-containing IgG were indistinguishable

Currie and Sime[15] Cells at 5×10^7 ml^{-1} in MEM were taken into capillaries which were then sealed at one end with wax After centrifuging the cells towards the seal each capillary was cut at the cell–medium interface, and the open end of the cell-containing part was attached with silicone grease to the base of a culture well in a Sterilin S25 plastic plate The wells were filled with antibody or control serum and sealed with cover slips Migration into the medium took place for 16 h at 37 °C Relative areas of migration were estimated by projecting the cell patterns on to paper, cutting round the edges and weighing The results (Fig 3) reveal a significant degree of inhibition by the antibody serum

To confirm that the tumour-specific antibody is in fact directed against cell surface Ig, we took advantage of the molecular discrimination of the capping-pinocytosis phenomenon[14], whereby two sets of antigenic determinants cap simultaneously if on the same molecule, but otherwise independently In this case, antibody known to react with the surface Ig (in fact with C region determinants on the light chain) capped the determinants with which the tumour-specific antibody reacted and vice versa For the first experiment L_2C cells were suspended at 2×10^7 ml^{-1} in rabbit anti-guinea pig Fab'γ serum, 50% v/v in Eagle's MEM, and incubated at 37 °C for 30 min At the end of this period capping and internalisation were essentially complete, as indicated by the behaviour of an aliquot of cells allowed to react with fluorescein-conjugated IgG from the same antiserum The cells were chilled and washed, and without being rewarmed, were shown to have lost virtually all their reactivity with the tumour-specific antibody serum in the indirect immunofluorescence test In contrast a sheep anti-guinea pig lymphocyte serum retained full reactivity with the treated cells For the second experiment it was first found that incubation in tumour-specific antibody serum failed to give any capping on the L_2C cells, presumably as a result of the low antibody concentration in this serum A second layer of anti-antibody on the cell could, however, induce capping the cells were allowed to react at 4 °C for 30 min with antibody serum and then, after washing, were incubated at 37 °C for 30 min with a rabbit anti-sheep IgG serum which had previously been absorbed with guinea pig Ig Again examination of an aliquot of cells treated in the second incubation with a fluorescein conjugate of the anti-antibody suggested that

capping and internalisation were essentially complete at the end of the 30-min period The chilled, washed cells now showed greatly reduced reactivity with fluorescein-conjugated IgG from rabbit anti-guinea pig Fab'γ Cells treated similarly with control serum for the first step, showed the usual bright speckled fluorescence with fluorescein-anti-Fab'γ

We suggest that the availability of antisera to a tumour-specific molecule which can be identified and quantified, and which appears in the cellular environs simply as a result of turnover at the cell surface, is of considerable research and therapeutic interest An approach similar to that used here may be appropriate for a variety of tumour-specific antigens

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Thymus reactive IgM autoantibodies in normal mouse sera

An important characteristic of the immune system is the ability to discriminate between antigens expressed on normal tissues within the individual and the many foreign antigens expressed on normal tissues of other species (xenoantigens) and even on normal tissues of members of the same species (alloantigens) Thus, although the immune system readily destroys inoculated xenogeneic and allogeneic tissue, autoimmune damage to normal, autologous tissue is rare The rejection of inoculated foreign tissue is thought to reflect an immune surveillance mechanism by which an individual could detect and eliminate his own aberrant cells expressing abnormal surface antigens[1] This mechanism may be important in restricting the development of many nascent tumours[1-3] Little is known, however, concerning either the mechanism by which the immune system destroys nascent tumours or the mechanism preventing the immune system from inflicting autoimmune disease The finding of near normal tumour incidence in congenitally athymic (nude) mice has suggested that immune surveillance may not involve the activity of thymus-dependent immunity but rather be a function of humoral immunity[4] In this respect it is interesting that sera of both normal and nude mice contain antibodies reactive with a wide variety of tumour cells[5,6] There is no evidence, however, that these antibodies function in the destruction of nascent tumours or that they are necessarily directed against tumour specific rather than normal tissue associated antigens It has been demonstrated that naturally occurring antibodies (NOA) reactive with the neural tumour cell line NB1 can be specifically absorbed by syngeneic normal brain tissue[6] We demonstrate further the autoantibody nature of certain NOA by documenting the occurrence in normal mouse sera of IgM antibodies reactive with autologous thymus cells

Sera of C3H/HeJcrf mice (C3H) were tested in the trypan

blue dye exclusion cytotoxicity assay for complement dependent cytotoxic activity against syngeneic thymus and spleen cells In 9 out of 10 experiments, serum of normal C3H mice caused significant lysis of syngeneic thymus cells (Table 1) In 12 experiments, the percentage lysis of syngeneic spleen cells by normal C3H serum varied from -54% to 73% with a mean specific lysis of -26% The anti-thymus cytotoxic activity of normal C3H serum was rabbit complement (RC') dependent since lysis of thymus cells was not observed if the rabbit serum used in the assay had been heat inactivated at 56 °C for 30 min or if guinea pig serum, adequate for the detection of anti-θ^{C3H} antibodies, was substituted for rabbit serum as a source of complement The anti-thymus cytotoxic activity of normal C3H serum was attributed to naturally occurring IgM antibodies since the cytotoxicity could be absorbed from mouse serum with anti-mouse immunoglobulin antibody-bound sepharose beads, and by a monospecific preparation of goat anti-mouse IgM antibody The cytotoxic activity was heat labile and susceptible to 0 1 M 2-mercaptoethanol In addition, when mouse serum was chromatographed on a Sephadex G-200 column, RC' dependent cytotoxic activity was detectable only in the excluded fraction (Table 2)

Undiluted normal sera of various strains were tested for RC' dependent cytotoxicity against autologous thymus cells Sera of all strains tested were cytotoxic for autologous thymus cells (Table 3) Repeated experiments on mice obtained from

Table 1 Complement dependent cytotoxicity of serum of C3H mice against syngeneic thymus cells

Experiment no	Lysis resulting from RC' (%)	Specific lysis (%) resulting from normal serum plus RC'
1	2 3	38 9
2	6 2	26 2
3	8 3	6 3
4	5 7	47 0
5	12 8	52 8
6	4 7	24 6
7	3 7	36 4
8	8 5	39 5
9	3 2	23 7
10	4 1	22 9

Mice 3–6 months old were bled from the tail vein The serum was separated by centrifugation approximately 1 h later and either tested directly or stored in aliquots at -20 °C Cell suspensions were prepared by gentle teasing of the thymuses in Dulbecco's modified Eagle's MEM containing 10% foetal calf serum (DMEM-10) followed by filtration through 100-mesh nylon net In the trypan blue dye exclusion cytotoxicity assay, 50 µl of a suspension containing 3×10^6 cells per ml in DMEM-10 were added to 50 µl of normal mouse serum The mixture was incubated for 60 min in a humidified incubator at 37 °C in a CO_2-air (10 90) atmosphere The cells were washed with 300 µl of medium and resuspended in 50 µl of the optimal dilution of rabbit serum as a source of complement (usually a 1 4 dilution) The rabbit serum had been absorbed previously at 2 °C with a one-third volume of packed thymus and spleen cells to reduce its toxicity Several batches of rabbit serum were used in these experiments Sera from some rabbits were excluded, however, because of either residual toxicity or low complement activity After a 60-min incubation at 37 °C, tubes containing the cells and RC' were placed in an ice bath A volume of 50 µl of trypan blue solution was added to the cell suspension and a sample was removed for microscopic enumeration of the percentage non-viable (stained) cells At least 100 cells were counted in each determination Controls included in each experiment were (1) incubation of cells with normal mouse serum followed by the addition of medium rather than RC' (antibody control), and (2) initial incubation of the cells in medium followed by incubation in RC' (RC' control) The antibody control value was invariably < 5% The RC' control value is indicated in the table In other experiments, unless otherwise indicated, the RC' control value was < 10% Duplicate determinations were performed The correlation observed between duplicate determinations was such that a calculated value of > 10% specific lysis was significant The 10 sera used in the experiments presented in this table were obtained from groups of 3–5 mice, 2–6 months old Specific, mouse serum induced lysis was calculated by

$$\frac{(\% \text{ Cytotoxicity (mouse serum} + \text{RC')} - \text{RC' control}) \times 100}{100 - \text{RC' control}}$$
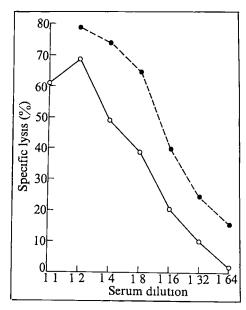

Fig. 1 Complement dependent anti-thymus cytotoxicity of sera obtained from nude (●) and normal C3H (○) mice The titration curve for nude and normal mouse sera represents the mean of values obtained using three distinct pools of sera from both nude and normal mice

the Imperial Cancer Research Fund and from the Rodent and Rabbit Production Section of the National Institutes of Health have confirmed that sera of many mouse strains regularly (> 80% incidence) contain readily detectable levels of thymus reactive cytotoxic autoantibodies Mouse sera are generally cytotoxic for allogeneic thymus cells, although thymus cells from different strains vary in their susceptibility to lysis by a given allogeneic serum (Table 3) Sera from nude mice possess significant levels of NOA reactive with allogeneic thymus cells (Fig 1) To determine whether susceptibility to

Table 2 Evidence for IgM antibody nature of naturally occurring anti-thymus cytotoxic activity in normal C3H serum

Experiment no	Serum treatment	Specific (%) lysis resulting from normal C3H serum plus RC'	
		Control serum	Treated serum
1	Heated (60 °C)	38 5	8 8
2	2-ME	33 0	2 2
3	Incubated with GAM IgM*	23 7	0 3
4	Absorbed with Seph-RAMIg†	56 3	12 2
5	Chromatographed on Sephadex G-200‡		
	Unfractionated	73 1	
	1st peak (IgM)		79 2
	2nd peak (IgG)		3 4

The trypan blue dye exclusion cytotoxicity assay was performed and the specific (%) lysis calculated as described in the footnote to Table 1 Equal volumes of normal mouse serum and 0 2 M 2ME in phosphate-buffered saline were incubated at 37 °C for 45 min Control serum was diluted with phosphate-buffered saline and similarly incubated

*Monospecific goat anti-mouse IgM antibody was provided by Dr M Cooper 500 µg of anti-mouse IgM antibody in 100 µl balanced salt solution (BSS, pH 7 2) was mixed with an equal volume of normal mouse serum and incubated at 25 °C for 3 h Normal mouse serum diluted with an equal volume of BSS served as a control

†Sepharose-bound purified rabbit anti-mouse immunoglobulin antibody (Seph-RAMIg) was provided by Dr D Kilburn Normal serum was incubated with either Sepharose or Seph-RAMIg and incubated at 25 °C for 1 h After centrifugation sera were tested for anti-thymus cytotoxic activity

‡2 ml of serum was applied to a Sephadex G-200 column Protein contained in the void volume (1st peak) and the 2nd peak was concentrated to 2 ml by Amicon filtration and shown by immuno-electrophoresis to react specifically with rabbit anti-mouse IgM and rabbit anti-mouse IgG antisera, respectively

Table 3 Complement dependent cytotoxicity of normal mouse sera against autologous and allogeneic thymus cells

Mouse strain	Lysis resulting from RC' (%)	Specific (%) lysis resulting from autologous serum plus RC'	Specific (%) lysis resulting from normal C3H serum plus RC'
C3H	1 0	22 9	36 0
C57BL	1 0	29 2	46 3
A	2 5	20 3	29 1
BDF$_1$	1 3	30 7	26 0
Hairless	17 9	21 0	16 8
DBA/2	4 4	50 0	ND
129	23 2	74 2	ND
Schneider	19 9	52 8	ND
NZB	12 6	76 3	ND
C57BT	9 2	31 6	ND

ND, not determined

lysis by NOA was confined to functionally immature thymocytes, normal sera of C3H mice were tested for cytotoxicity against thymocytes obtained either from hydrocortisone-treated adult mice or from neonatal mice As Table 4 shows, thymus cells of hydrocortisone-treated mice were susceptible to lysis by NOA Thymus cells obtained from neonatal mice were lysed but only by undiluted normal serum

Schlesinger found thymus reactive autoantibodies in sera of all strain 129 mice 5 weeks or older[8] Shirai and Mellors, however, detected thymus reactive antibodies in only 46 out of 120 strain 129 mice 2–11 months old[9] In a survey of 10 other strains Shirai and Mellors regularly detected antibodies reactive at 37 °C with thymocytes in sera of 2–11 month old mice of the NZB strain (69 out of 97 sera tested) Anti-thymus antibodies were detected in a small proportion of normal sera obtained from several other strains, including C58 (17 out of 40 sera tested), and NZW (6 out of 25 sera tested) and were occasionally present in sera of AKR (14 out of 120), BALB/c (1 out of 29), C3HeB/Fe (3 out of 30), C57BL/6 (2 out of 90) and SJL (1 out of 21) strain mice Thymus reactive cytotoxic antibodies were not detected in 23 sera of DBA/2 mice or in 20 sera of A mice Boyse et al[10] detected thymus reactive autoantibodies in several mouse strains but only after the mice were immunised with allogeneic thymus cells These reports stand in contrast to that of Raff who, using hamster serum as the source of complement, detected high titres of thymus reactive antibodies in sera of all strains tested[11] Our findings confirm and extend

Table 4 Reactivity of normal serum of C3H mice against thymus cells of hydrocortisone-treated adult and untreated neonatal C3H mice

Thymus cell donor	Specific (%) lysis resulting from 1 1 and 1 2 dilutions of C3H mouse serum plus RC'	
	1 1	1 2
Normal adult	58 6	64 4
Hydrocortisone-treated adult*	41 7	49 4
Normal neonatal†	33 8	−2 9

*Six week old mice received 5 mg hydrocortisone acetate intraperitoneally 48 h before removal of their thymus Less than 10^7 thymocytes could be obtained from each hydrocortisone-treated mouse, whereas approximately 10^8 thymocytes were obtained from each normal mouse
†Mice less than 24 h old were used as thymus donors

those of Raff The failure of many investigators to detect thymus reactive autoantibodies in normal mouse sera may be the result of their use of inadequate amounts of complement or of complement with a high level of residual cytotoxicity due to inadequate absorption with normal tissue Thus, for example, when RC' was used at a lower than optimal concentration cytotoxic anti-thymus antibodies were detected in sera of 129 and NZB mice but not in sera of C3H, C57BL or A mice (data not shown)

The finding of thymus reactive antibodies in sera of nude

mice indicates that the thymus is not required as the antigenic source for the production of such antibodies and that the antibody response in normal mice is probably thymus independent Absorption studies have indicated that several thymus antigens are recognised by NOA. The nature and tissue distribution of individual antigens are being analysed The observation that hydrocortisone-resistant thymus cells are susceptible to lysis by NOA indicates that even within the thymus many of these antigens are not restricted to functionally immature thymocytes Whether the cells which actually leave the thymus continue to express these antigens has not been confirmed In any event the injection of thymocytes directly into the circulation provides an experimental system with which to study the consequence of the interaction of NOA and target cell and may help to elucidate the biological function of tumour reactive NOA

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Carrier preimmunisation in the anti-hapten response of a marine fish

A STRONG antibody response in mammals and birds requires collaboration of two types of antigen-recognising lymphocytes, T and B Only the B lymphocyte gives rise to antibody-producing plasma cells This concept is partly based on the observed synergistic effect of thymic (non-B) cells and bone marrow (non-T) cells in antibody responses[1-3] Another line of evidence comes from immunisation with an antigen having several antigenic determinants such as A–B–C–D, which can be separated into portions A strong synergistic effect in an antibody response against A is found by combining lymphocytes primed with one part of the antigen (B–C–D = carrier) with lymphocytes primed with another part (A = hapten) and challenging the mixture with A–B–C–D (refs 4 and 5) T lymphocytes seem to be the relevant cells in the carrier-primed population[6]

Whether the collaboration of two types of antigen-recognising lymphocytes is also important for antibody responses of lower vertebrates is not known Studies of fishes, amphibia and reptiles are handicapped by the lack of histocompatible strains This excludes cell transfers from one individual into another, which have been the basis of all the studies cited above and makes *in vitro* collaboration experiments in lower vertebrates unattractive since histocompatibility between collaborating cells may be necessary for a good response *in vitro*[7] A phenomenon which can strongly suggest importance of cell collaboration and does not involve cells from more than one individual animal is an enhanced anti-hapten response caused by carrier preimmunisation This has been shown to correlate well with cell transfer data in mammals and birds[8-11], and we have used this method in the winter flounder (*Pseudopleuronectes americanus*)

Winter flounder, weighing between 0 2 and 0 3 kg, were caught using an otter trawl in Sandy Hook Bay, and were

Table 1 Anti-NIP response in the winter flounder

Group	No of fish	Priming	Challenge	Log mean anti-NIP titre‡ ±s e
A	9	CG–FCA*	$NIP_{13}CG$†	5 534±0 190
B	7	Saline-FCA	$NIP_{12}CG$	4 344±0 163
C	16	None	None	4 435±0 062

*Fishes were primed with 1 mg kg^{-1} chicken globulin (CG) in Freunds complete adjuvant (FCA)

†4-Hydroxy-3-iodo-5-nitrophenyl acetyl conjuguted to CG (1 mol CG to 12 mol NIP 0 5 mg kg^{-1} in FCA)

‡Haptenated phage inactivation titre on day 21

P values for A against B or A against C < 0 001

Difference between B and C was statistically insignificant

kept in continuous flow salt water tanks in the range 16–18 °C The fishes were injected intraperitoneally with chicken globulin (1 mg per kg of body weight) in complete Freunds complete adjuvant (FCA), controls were given saline in FCA After 3 weeks both groups were injected with 0 5 mg kg^{-1} NIP-CG (Table 1) and bled for sera after 3 weeks by puncture of the dorsal aorta under anaesthesia with tricanemethane sulphate The antibody was detected by the haptenated phage inactivation test according to the method of Becker and Makela[12]

The results indicate that one injection of the conjugate did not cause a measurable anti-NIP response in 3 weeks unless the animals were preimmunised with the carrier protein (Table 1) Flounders which had been preimmunised by FCA alone had antibody titres indistinguishable from the level of natural anti-NIP in this species Carrier-preimmunised animals, on the other hand, had more than ten times greater titres than either of the other groups

Flounder sera were unexpectedly strong inactivators of NIP-T4 phage This inactivation may be caused by natural anti-NIP antibodies Flounder sera did not inactivate unconjugated T4 phage, and inactivation of the NIP-T4 was inhibited by 3 × 10^{-6} M NIP but not by 10^{-3} M DNP (highest tested concentration) Both haptens were in the form of protein conjugates (10–15 mol hapten per mol BSA)

Our finding suggests that in fishes carrier-recognising cells also collaborate with hapten-specific cells for an optimal anti-hapten response As fishes are the most primitive major order of antibody forming animals, the available data suggest that collaboration of two types of recognising lymphocytes is a general requirement for the initiation of antibody production

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Symmetrical transcription of herpes simplex virus DNA in infected BSC-1 cells

ALONI[1-3] reported that late in the replicative cycle of polyoma and simian virus 40 (SV40) viruses the viral DNA genomes are symmetrically transcribed in the infected cells The newly synthesised RNA molecules labelled for very short periods were shown to be complementary and on self-annealing yielded high molecular weight double stranded (ds) RNA Aloni and Attardi showed that similar symmetrical transcription occurs in HeLa cell mitochondria[4,5] In further studies Aloni[2] demonstrated that after polyadenylation of the SV40 symmetrical RNA transcripts the RNA is processed to yield uncomplementary viral mRNA molecules

Since uninfected BSC-1 green monkey kidney cells which were used in our studies on herpes simplex virus (HSV) were found by Aloni[1] to have very small amounts of self-annealing RNA, we decided to study the nature of the transcription of HSV DNA, a linear genome with a molecular weight of 100×10^6 (ref 6) Studies by Roizman and his collaborators on the transcription of HSV DNA in infected nuclei revealed the synthesis of a high molecular weight precursor to viral mRNA[7,8] and the presence of polyadenylic acid [poly(A)] sequences in some of the virus-specified RNA[9] Wagner *et al*[10] reported that 90% of the HSV DNA genomes are transcribed late in the infection There is however no information on the mode of transcription and the DNA strand selection for transcription of HSV DNA Here we demonstrate that viral mRNA molecules which lack poly(A), transcribed from HSV DNA in BSC-1 cells late in infection, contain complementary sequences

BSC-1 monolayer cultures were infected with the HF strain of herpes simplex virus type 1 (ref 11) In these cells the HSV DNA is synthesised from 3 to 15 h after infection and the virus growth cycle is completed at 18 h The infected cells were pulse labelled with ^{3}H-uridine at 12 or 13 h after infection for 15 to 20 min, the RNA was extracted and analysed for complementary sequences by self annealing The amount of HSV specific RNA in the total RNA isolates does not exceed 10% as determined by hybridisation with HSV DNA

Figure 1a demonstrates the isolation of dsRNA molecules after self-annealing of labelled RNA HSV infected cells were labelled for 15 min at 13 h after infection and the total labelled

Table 1 Nuclease resistance of self-annealed RNA*

| Enzyme treatment | Trichloroacetic acid precipitable | | |
	^{3}H-self-annealed RNA %	^{3}H-rRNA %	^{3}H-HSV DNA
None	100	100	100
Pancreatic RNase	100	3	ND
Pancreatic RNase +DNase	100	ND	5
Denaturated RNA or DNA with RNase or DNase	5	5	12

*Total RNA from infected cells was prepared as indicated in Fig 1b Self-annealing was done in 4×SSC at 72 °C for 17 h and the self-annealed RNA peak was eluted from the Sephadex G-50 column after RNase (25μg ml⁻¹) treatment RNase treatment was in 0 2 M NaCl, 10 mM Tris-HCl, pH 7 4 10 mM EDTA at 37 °C for 60 min with 2,000 c p m of self-annealed RNA or 2,000 c p m ribosomal RNA (rRNA) DNase treatment was with 2,000 c p m HSV DNA in 100 mM NaCl, 10 mM MgCl₂, 10 mM Tris-HCl pH 7 4 at 37 °C for 60 min For the simultaneous incubation of RNase and DNase, 10 mM MgCl₂ (final concentration) was added to the RNase buffer without EDTA Thermal denaturation was at 100 °C for 5 min after which the samples were cooled in ice water The trichloroacetic acid (TCA) precipitable material was determined
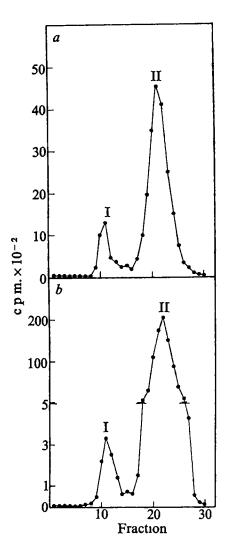
ND, not done

Fig. 1 Exclusion chromatography of self-annealed, RNase-treated RNA on a column of Sephadex G-50 *a*, Total RNA, $1\,5\times10^7$ BSC-1 cells were infected with HSV (10 PFU per cell) and incubated at 37 °C for 13 h The infected cell cultures were then labelled with ^{3}H-uridine for 15 min (200 µCi ml^{-1}, specific activity 29 Ci mM^{-1}) The cells were chilled in ice at the end of the labelling period and scraped into RSB buffer (10 mM Tris-HCl *p*H 7 4, 10 mM NaCl, 1 5 mM MgCl$_2$) The swollen cells were homogenised in a tight glass Dounce homogeniser (30 strokes) Total labelled RNA was extracted twice with a mixture of phenol and chloroform 1 · 1 (v/v) at room tempereture and twice with chloroform–isoamyl alcohol (99 1) The RNA was precipitated in ethanol, dissolved in DNase buffer (10 mM Tris-HCl *p*H 7 6 100 mM NaCl, 10 mM MgCl$_2$) and incubated with DNase (50 µg ml^{-1}) for 15 min at 37 °C The RNA was extracted once more as above and was brought to 60% formamide and 2×SSC (1×SSC = 0 15 M NaCl, 0.01 M sodium citrate) and annealed by incubation at 37 °C for 24 h The RNA was precipitated with ethanol and resuspended in RNase buffer (0 2 M NaCl, 10 mM Tris-HCl *p*H 7 6 and 10 mM EDTA) and treated with RNase (5 µg ml^{-1}) at 37 °C for 60 min The RNA was diluted with the application buffer (0 1 M NaCl, 0 1% sodium dodecyl sulphate (SDS) 2 mM EDTA, 10 mM Tris-HCl, *p*H 7 7) and applied to a 100 cm Sephadex G-50 column Thirty fractions of 2 5 ml each were collected and 25 µl samples were used to determine the radioactivity in each fraction Peak (I) is the self-annealed RNA and peak (II) contains the low molecular weight RNA products, which remain after RNase treatment *b*, Exclusion chromatography of self-annealed, RNase treated RNA which did not bind to a cellulose column (poly(A) minus RNA) HSV infected cells at 12 h after infection were pulse-labelled for 15 min with ^{3}H-uridine (200 µCi ml^{-1}) Total RNA was prepared as in *a* After DNase treatment and phenol extraction, the aqueous phase was diluted with the application buffer containing 0 5 M KCl, 10 mM Tris-HCl *p*H 7 6 and 0 2 mM MgCl$_2$ The solution was applied to a cellulose column using the technique of Schutz *et al* [12] The RNA was washed with the application buffer (0 5 M KCl, 0 2 mM MgCl$_2$, 0 01 M Tris-HCl *p*H 7 6) and the unbound RNA was eluted After extensive washing with the buffer, when no more labelled RNA was eluted the cellulose
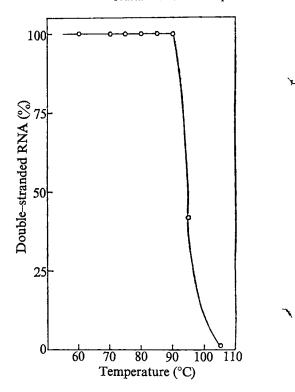

Fig. 2 RNase susceptibility of self-complementary RNA as a function of the denaturation temperature HSV-infected BSC-1 cells were labelled with ^{3}H-uridine (200 µCi ml^{-1}) for 20 min at 13 h after infection Total RNA was extracted as described in the legend to Fig 1*a* and self-annealed for 17 h at 72 °C in 4×SSC buffer The preparation was RNase treated (30% of the radio-activity was RNase resistant) and chromatographed on a Sephadex G-50 column as in Fig 1*b* The labelled RNA which was eluted in the first peak (as shown in Fig 1) was ethanol precipitated overnight and resuspended in 0 1×SSC Samples (each containing 1,600 c p m) from the dsRNA peak were distributed into glass tubes in 0 1×SSC and heated for 5 min at the indicated temperatures and rapidly cooled The samples were then brought to 2×SSC and treated with 50 µg ml^{-1} pancreatic RNase for 60 min at 37 °C The samples were TCA precipitated and the radioactivity was determined

Table 2 Hybridisation of denatured dsRNA with HSV DNA

^{3}H-RNA source	DNA source	Input (c p m)	Hybridised (c p m)	%
Denatured dsRNA	HSV	2,000	450	22 5
	BSC$_1$	2,000	86	4 3
	—	2,000	75	3 7
Cellular RNA	HSV	50,000	450	0 9
	BSC$_1$	50,000	1,600	3 2
	—	50,000	85	0 17

^{3}H-labelled dsRNA was obtained from the infected cells used for the experiment described in Fig 1 The dsRNA was denatured by heating for 5 min at 100 °C, cooled rapidly and hybridised in solution to 30 µg ml^{-1} of alkali-denatured HSV DNA or 350 µg ml^{-1} of alkali-denatured BSC$_1$ DNA The HSV DNA was purified from herpes simplex virions isolated in sucrose gradients The hybridisation was carried out in a buffer containing 0 4 M NaCl, 1 mM Tris-HCl, *p*H 7 4, 0 5 mM EDTA, final volume 100 µl for 24 h at 72 °C At the end of the hybridisation each sample was treated with RNase (50µg ml^{-1} for 60 min at 37 °C) and the hybrids were trapped on Millipore filters The filters were washed with 2×SSC and the radioactivity determined

column was washed with neutralised H$_2$O to elute the poly(A)-containing mRNA which was retained on the column The unbound RNA species which were eluted with the application buffer were used for self-annealing in 4×SSC for 17 h The samples were treated with 25 µg ml^{-1} RNase in RNase buffer as in *a* The RNA solution was chromatographed on a Sephadex G-50 column as in *a* Peak I is the self annealed RNA and peak II contains the low molecular weight RNA products, which remain after RNase treatment

RNA was extracted Similarly, dsRNA molecules were obtained from a ³H-RNA preparation which was chromatographed on a cellulose column to isolate the poly(A) containing RNA molecules by the technique of Schutz *et al*[12] In these conditions 12% of the total labelled RNA was bound to the column About 27% of the labelled RNA molecules synthesised in the nuclei of HSV infected BSC-1 cells are complementary and can form double-stranded RNA molecules, resistant to RNase treatment Further studies on the self-annealing of the poly(A) containing labelled RNA are in progress

We studied further the properties of the dsRNA molecules Heating as described in Fig 1 resulted in the denaturation of the dsRNA molecules and rendered them sensitive to RNase treatment (Fig 2) The dsRNA underwent a sharp transition in nuclease resistance at 95 °C in 0 1 × SSC, a higher temperature than SV40 dsRNA[1,2] The dsRNA was completely resistant to RNase and to a mixture of RNase and DNase at
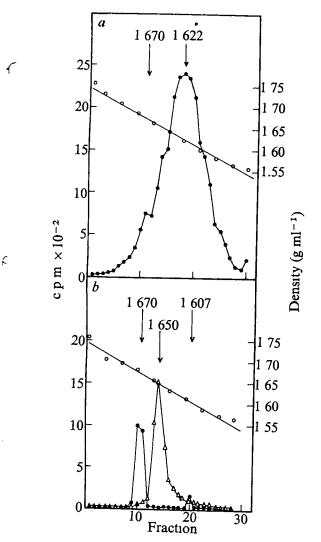

Fig 3 Buoyant density of self-complementary RNA in Cs_2SO_4 gradients HSV infected BSC-1 cells, at 12 h after infection were labelled with ³H-uridine (200 μCi ml⁻¹) for 15 min and the total RNA was extracted as described in the legend to Fig 1*a*, self annealed RNase treated and eluted from a Sephadex G-50 column The RNA preparation was divided into two portions One was left untreated (*a*) and the second was heated (4 min at 100 °C) and quickly cooled (*b*) To the two ³H-RNA preparations in buffer (0 05 M Tris-HCl, *p*H 7 4, 0 005 M EDTA), Cs_2SO_4 crystals were added to an initial density of 1 580 g ml⁻¹ To gradient (*b*) ¹⁴C-labelled ribosomal RNA was added as a density marker The tubes were centrifuged at 35,000 r p m in the Beckman ultracentrifuge at 20 °C in the rotor 50 Ti for 46 h The gradients were collected dropwise from the bottom and the density and the TCA precipitable radioactivity in each fraction were determined Symbols *a*, ●——● self-annealed dsRNA, O——O density *b*, ●——● denatured self-annealed ³H-RNA, △——△ ¹⁴C ribosomal RNA marker, O——O density
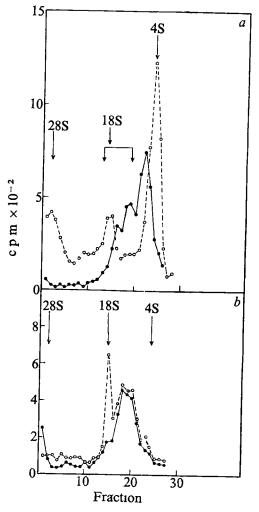

Fig 4 Sedimentation of dsRNA in sucrose gradients before and after denaturation *a*, dsRNA (●——●) was prepared as indicated in Fig 1 The dsRNA resuspended in SDS buffer after Sephadex G-50 filtration was layered onto a linear 5-20 (w/w) sucrose gradient in RSB containing 0 5% (w/v) SDS and centrifuged in SW41 rotor at 40,000 r p m , at 20 °C for 4 5 h Fractions were collected from the bottom of the tube dropwise and the TCA precipitable material was determined in each fraction ¹⁴C-labelled rRNA (O——O) served as internal marker *b*, Fractions 13–19 from the gradient (*a* arrows) were pooled, ethanol precipitated and resuspended in 10 mM Tris-HCl *p*H 7 4, 1 mM EDTA and divided into two portions, one (O) was denatured with formaldehyde at 70 °C as described by Aloni[1], the other (●) was kept untreated The samples were centrifuged in a 5-20 sucrose (w/w) gradient containing 0 5% (w/v) SDS in RSB in the SW41 rotor at 20 °C at 40,000 r p m for 4 5 h Fractions were collected dropwise and the TCA precipitable radioactivity was determined in each fraction, ¹⁴C-uridine-labelled rRNA served as internal marker

37 °C, but became completely sensitive to RNase after heating for 5 min at 100 °C (Table 1)

The labelled dsRNA molecules were centrifuged in Cs_2SO_4 and were found to band at a density of 1 622 g ml⁻¹ (Fig 3*a*) whereas after heat denaturation the RNA molecules banded at a density of 1 670 g ml⁻¹ (Fig 3*b*) The molecular size of the dsRNA molecules was determined by centrifugation in sucrose gradients (Fig 4) Some of the radioactive RNA sedimented to the position of 4S and some ranged from 10S to 16S Denatured RNA molecules also sedimented with a peak at approximately 18S, while the main mass sedimented at 8–10S (Fig 4*b*)

To determine the specificity of the dsRNA in respect to its homology with HSV DNA, the dsRNA molecules were isolated, denatured and hybridised in solution with denatured HSV DNA The dsRNA hybridised to HSV DNA and not to BSC-1 DNA (Table 2) It was noted that only 22 5% of the input RNA hybridised to HSV DNA This value may indicate that the

efficiency of hybridisation is low and that not all the RNA was HSV specific

Our study provides evidence that the primary transcription products in the nuclei of HSV-infected BSC-1 cells are transcribed from the two strands of the viral DNA in a symmetrical fashion The excluded peak from the Sephadex column is dsRNA as indicated by first, complete resistance to RNase at 37 °C, second, complete resistance to the simultaneous action of RNase and DNase, third, the sharp transition curve in nuclease resistance at 95 °C in $0 1 \times SSC$, which is not the melting temperature of the DNA–RNA hybrid, fourth, the density of 1 622 g ml^{-1} of the dsRNA which differs from the density of DNA–RNA hybrids, and finally the transition to the high density of 1 670 g ml^{-1}, which is specific for G+C rich DNA, on heat denaturation of the dsRNA

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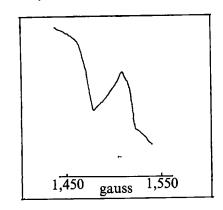*Jerusalem, Israel*

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Conversion of photoexcitation energy in rhodopsin

AN interaction between the 11-*cis*-retinylidene chromophore and nearest-neighbour molecules seems to be important for explaining the mechanism of conversion of photoexcitation energy, because the chromophore is enveloped by opsin It has been reported[1] that the time of the formation of prelumirhodopsin (lifetime of the excited state) is less than 6 ps

A weak electron spin resonance (ESR) signal for the forbidden transition $\Delta m = \pm 2$, as shown in Fig 1, was observed following

Fig. 1 ESR spectrum ($\Delta m = \pm 2$) of rhodopsin irradiated with visible light at 77 K The microwave frequency is about 9,230 MHz and the reproducibility of this ESR signal was about 70% Clear experimental conditions for inducing the triplet state were not discovered Free radicals ($g \simeq 2 00$, $\Delta H \simeq 18$ gauss) were also observed by ultraviolet irradiation (K S , unpublished)

1,450   gauss   1,550

the irradiation with visible light ($\lambda = 450$–600 nm) of frog rhodopsin extracted with 2% digitonin and mixed with an equal volume of glycerol so that it would freeze to a glass at 77 K A set of glass filters was used which cut out any light of less than 450 nm The intensity of illumination was $\sim$ 1,500 lx at the window of the cavity About one-tenth of the rhodopsin molecules (10^{14}–10^{15} unbleached rhodopsin molecules per effective volume) was transformed into the triplet state by irradiation This resonance signal disappeared after melting the irradiated rhodopsin and then freezing it again at 77 K The irradiated rhodopsin and then freezing it again at 77 K The solvent in the same glass tube without rhodopsin did not show such a resonance signal even after the same irradiation The important factors for this signal are the zero-field splitting parameters (D and E), because the spin orientation in the external magnetic field and the microwave frequency are known parameters of the experiments $D^*(=(D^2+3E^2)^{1/2})$ for rhodopsin is estimated from the magnetic field (H_{min}) of this signal as $0 117 \pm 0 002$ cm^{-1} The excitation of rhodopsin results in photochemical transformation of the 11-*cis*-retinylidene chromophore to all-*trans*[2], probably by way of the lowest triplet state T_1 (ref. 3) D for the retinal was evaluated by[4]

$$D = \tfrac{3}{4}g^2\beta^2 \sum_{p<q} (C_{ip}C_{jq} - C_{iq}C_{jp})^2 \times$$
$$\times \langle \chi_p(1)\chi_p(1)|r_{12}{}^2 - 3z_{12}{}^2/r_{12}{}^5|\chi_q(2)\chi_q(2)\rangle \qquad (1)$$

where β is Bohr magneton, C_{ip} and so on are the linear combination of atomic orbital (LCAO) coefficients, χ_p represents an atomic π orbital associated with the pth carbon atom, r_{12} is the distance from electron 1 to electron 2 and z is chosen parallel to the π orbitals of the retinal From equation (1), it is expected that the Hückel (H) and self-consistent-field coefficients are of the same order When D for the retinal was evaluated using the coefficient H, its value was about $0 057$ cm^{-1} E for the retinal is not zero, because of poor symmetry, but D^* for rhodopsin is greater than D^* for the retinal, so that E is generally chosen under the condition $|D| \geqslant 3|E|$ using the values of D (0 117 cm^{-1} and $0 057$ cm^{-1}) mentioned previously The triplet state was not therefore, the result of the conversion of the chromophore during isomerisation of 11-*cis* into all-*trans*, but was assumed to have arisen from photoexcited energy transfer from the chromophore to a molecule having π electrons, for example an aromatic amino acid of opsin The energy differences of the excited and the ground states of aromatic amino acids are,

Table 1 D^* values and linewidths, ΔH, of aromatic amino acids

Aromatic amino acids	$D^*=(D^2+3E^2)^{1/2}$ (cm^{-1})	ΔH (gauss)
L-Tyrosine		
Non-ionised	0 161†	}(18)
Ionised	0 141†	
Tryptophan	0 117±0 001‡	28
Tryptophyl-tyrosine	0 119±0 001‡	20
Phenylalanine	0 154±0 001‡	—
Rhodopsin	0 117±0 002	30

†Ref 6
‡Calculated values from data of refs 7 and 8

however, higher than those of the chromophore In this system, the inference is that formation of a hydrogen bond between the chromophore and an amino acid is necessary for this energy transfer, that is a charge transfer It may be suggested that the changes of λ_{max} of rhodopsin which is denatured by trichloroacetic acid[5] are attributable to the hydrogen bond between the chromophore and the amino acid The mechanism of the charge

transfer is probably induced by the electron transfer interaction between the π systems of the chromophore and of the amino acid The σ–π interaction of the hydrogen bond, $=\overset{+}{N}–H \cdot$, may be an exchange interaction, presumably involving the proton transfer. The wave function of the retinylidene chromophore may be written as

$$\Psi \simeq C_1\varphi_0 + C_2\varphi_{CT} \qquad (2)$$

where φ_0 and φ_{CT} mean the states containing the hydrogen bond and the charge transfer structure between the chromophore and the amino acid, respectively The observed signal is not attributable to the intermolecular triplet state, because D for rhodopsin is large We compared the D^* of aromatic amino acids and of rhodopsin (see Table 1), these results suggest that the ESR signal arises from the triplet state of tryptophan Although the process (CT complex→triplet state of tryptophan) is not yet known, it is possible that the triplet state of the tryptophan arises by way of a metastable state because the lifetime of the excited state of rhodopsin is short[1]

The mechanism of the charge transfer seems to be an important clue in clarifying the problems of the early receptor potential[9–10] which may be generated by charge separation in the rhodopsin molecule[11]

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NH₂-terminal amino acid sequence of the Fc fragment of IgD resembles IgE and IgG sequences

THE function of IgD, a rare immunoglobulin (Ig) class, is unknown[1] It has been shown that most B lymphocytes in cord blood[2] and lymphocytes of patients with chronic lymphatic leukaemia[3] carry IgD on their surfaces, whereas only a small portion of lymphocytes from healthy human adults is IgD-positive[4] Almost all lymphocytes having IgD on the surface also carry IgM and both seem to function as receptors[5] To learn more about the relationship of IgD to other Ig classes, we are investigating the amino acid sequence of the IgD Fc fragment This report describes our first sequence analysis which suggests that IgD is more closely related to IgE and IgG than to IgM

The NH₂-terminal amino acid sequence of the Fc fragment is shown in Fig 1 Two preparations of Fc fragments of protein Di and one of protein Ac were analysed for 42 steps and one preparation of Ac for 53 steps All preparations showed a single sequence starting with the residue Thr, and the Fc fragments of proteins Di and Ac were alike The first 13 amino

acids had a sequence identical to that reported for the peptide of the δ chain that contains the half-cystine residue forming the sole inter-heavy–heavy chain disulphide bond in IgD (ref 8) allowing us to place the determined sequence at the beginning of the Fc fragment The sequence in this area of the δ chain was characterised by a relatively high content of aromatic amino acids One residue each of histidine, tyrosine and tryptophan was found in positions 7, 14 and 24, respectively, and two phenylalanine residues in positions 31 and 34 In addition, there was probably a tryptophan residue in position 52 and a tyrosine

Fig 1 NH₂-terminal amino acid sequence of IgD Fc fragment of myeloma proteins Ac and Di The Fc fragments were isolated after digestion of IgD with trypsin for 2 min[8] We applied 300 to 600 nM into the cup of an updated Beckman Model 890B automated sequencer using the DMAA program No 11374 The cleaved amino acids were identified either by gas chromatography or automated amino acid analysis after hydrolysis with 6 N HCl or HI as previously described[7] The protein was not fully reduced and alkylated with ¹⁴C-iodoacetamide to determine half-cystine residues because such preparations were insoluble and unsuitable for sequence analysis The half-cystine residue in position 4 was recovered as alanine after HI hydrolysis and the residue in position 33 was assumed to be a half-cystine because of the absence of any recoverable residue at this position and homology to the other Ig Fc fragments The tryptophan residue at position 24 was identified by gas chromatography and as an increase of glycine after hydrolysis with HI Position 52 was probably also tryptophan because there was no residue other than a slight increase in glycine after HI hydrolysis No residue could be identified in position 47 The extrapolated initial yield of the Fc preparation (Ac) that could be sequenced for 53 steps was 296 nM, approximately 600 nM had been applied to the cup The average loss per step determined during the first 20 steps was 3 8% The yields over background after step 35 varied slightly from step to step and were decreasing gradually from 30 to 4 nM as in the case of tyrosine in step 53

residue in position 53 because three other Ig classes have a tryptophan residue in the analogous position, and no other residue was detected in this position and at step 53 tyrosine was detected in about three times the background The yields of amino acids after step 35 were low, and therefore the sequence after step 35 is less reliable No residues could be identified in position 47 Although low yields in the late steps may have led to erroneous identifications, the residues that could be identified are reported because they demonstrate the great degree of homology among the δ chain and the other
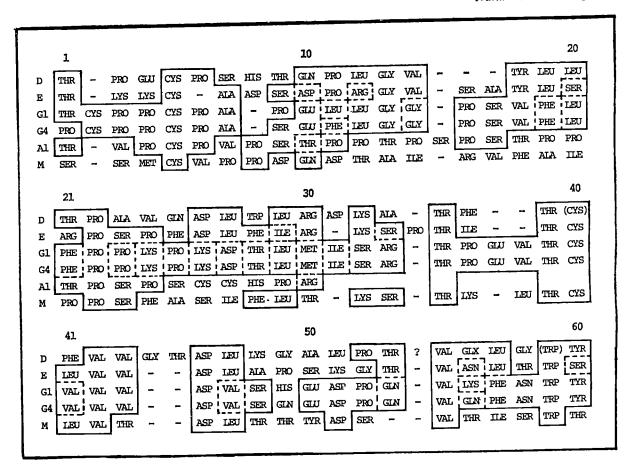
Fig. 2 Comparison of amino acid sequence of the IgD Fc fragment with the analogous sequences of other Ig heavy chains[9-13] Gaps were inserted to achieve maximal homology among all six proteins that were compared

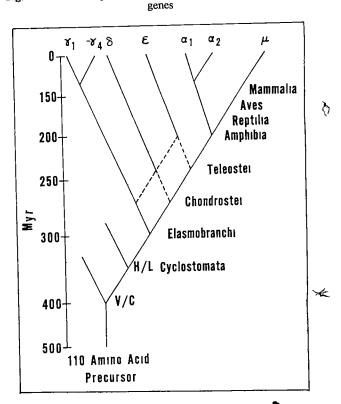heavy chains whether determined in sections at the beginning or end of the sequenced Fc portion

The δ chain and the other Ig heavy chains were strikingly homologous (Fig 2) when the sequences at the beginning of the Fc fragment that contains the half-cystine residue involved in the inter-heavy-heavy chain disulphide bond were compared Homologies with IgE (ref 9) (41%) closely followed by IgG1 (ref 10) and IgG4 (ref 11) (34%) were greatest, whereas IgM (ref 12) showed the least homology (23%) Relatively little is known about the amino acid sequence of IgA in this area, nevertheless, a hinge peptide of IgA1 (ref 13) had a reasonable degree of homology (24%) and was probably also derived from the beginning of the Fc fragment The degree of homology to any given Ig class was similar over the entire area of the Fc fragment that was sequenced It is therefore most likely that the remaining portion of the δ chain's constant region will show a similar degree of homology to the other heavy chains

Little information is thus far available on the COOH-terminal domain of the δ chain COOH-terminal analyses suggested the sequence Pro-Gly-COOH (ref 6) similar to that of IgG (Pro-Gly-COOH) and IgE (Pro-Gly-Lys-COOH) and contrasting to those of IgM and IgA (Cys-Tyr-COOH) Like the ε chain, the δ chain lacked a methionine residue near the COOH-terminus[6] that was found in γ, α and μ chains IgD has a high carbohydrate content[6] resembling that of IgE, IgA and IgM and differing markedly from that of IgG

Based on these data and resemblance of the different heavy chains to one another I have attempted to place the δ chain into an evolutionary tree that was previously proposed for the other Ig polypeptide chain genes[14,15] (Fig 3) The relatively large difference in structure between the δ chain and the other heavy chains suggests that it evolved rather early Because γ and

μ chains differ the most, they probably diverged first, perhaps about 300 Myr ago[15] The δ chain and ε chain probably

Fig. 3 Scheme of possible evolution of Ig polypeptide chain genes

evolved next, either from a primitive γ- or μ-like heavy chain, 200-300 Myr ago (before the appearance of mammals) The time span of the appearance could be anywhere between 200 and 300 Myr ago, because it has been estimated that the ɣα chain which shows greater homology to the μ chain evolved about 200 Myr ago Obviously, phylogenetic studies of IgD will also be necessary to place evolutionary origin of IgD with greater certainty. If as predicted, however, IgD evolved before mammals appeared, one should be able to find an Ig class analogous to human IgD in lower vertebrates such as teleosts, amphibians, reptiles and birds

Unfortunately, IgD is only a minor serum component[1], and it has been extremely difficult to isolate it from serum without a specific antiserum Since IgD seems to be a lymphocyte receptor, however, an approach recently used to demonstrate an IgD analogue in mice[16,17] could be applicable to the problem These investigators first labelled the lymphocyte surface Ig and precipitated the IgM with a μ chain specific antiserum, in a second step they precipitated any other Ig that was present on the lymphocyte membrane with an anti-light chain antiserum An Ig with characteristics resembling IgD was then discovered Attempts to demonstrate this IgD-like substance on foetal murine lymphocytes were unsuccessful, but IgM was demonstrable by this method, suggesting that in ontogeny IgM appeared before IgD Since ontogeny often parallels evolution, this finding could corroborate the position of IgD proposed in Fig 3

Although the function of IgD is still unknown, an early appearance and persistence in evolution would suggest its importance in the immune system The striking resemblance of IgD to IgE and IgG, both known to have cytophilic properties[18], led us to study the binding of IgD myeloma proteins to lymphocytes, neutrophils and monocytes, however, no binding to any of these cell types could be demonstrated[19] It seems, therefore, most likely that IgD is a lymphocyte receptor the function of which may be quite different from that of IgM, to which it bears the least homology

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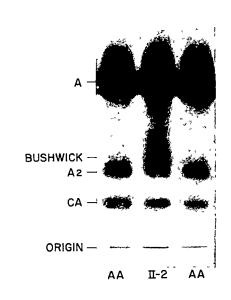[19] Lawrence, D A, Weigle, W O, and Spiegelberg, H L, J clin Invest, 55, 368–376 (1975)

Rapid postsynthetic destruction of unstable haemoglobin Bushwick

MOST structural variants of human haemoglobin occur in smaller amounts than HbA in the peripheral blood of heterozygous subjects Abnormal haemoglobins with mutations in the β chain usually comprise 35–45% of the haemoglobin in haemolysates In contrast, haemoglobins with abnormal α chains generally amount to only 20–25% of the total haemoglobin This difference in the proportion of α and β chain variants is thought to reflect the fact that in some populations there are two α loci and only one out of four α globin genes is affected in a heterozygote, whereas one out of two β globin alleles is abnormal in a subject with a β variant[1,2] Certain unstable haemoglobins with critical alterations in β-chain structure also constitute a much smaller proportion of the total circulating haemoglobin than HbA The low concentration of these abnormal haemoglobins is the result of accelerated preferential destruction[3-7] We report here a new unstable β variant, Hb Bushwick, which was detected in an intact form constituting only 1–2% of the total circulating haemoglobin Evidence was found for rapid postsynthetic destruction of the abnormal β chain In spite of this accelerated loss of the product of one of the two β alleles, the affected erythrocytes contained normal amounts of haemoglobin

The variant haemoglobin was discovered during the investigation of an Italian–American woman with a mild chronic anaemia and a history of several episodes of moderately severe haemolysis and jaundice associated with drug administration and intercurrent infections The presence of an unstable haemoglobin was suggested by the generation of multiple, small, coccoid, intraerythrocytic inclusion bodies on incubation of the subject's blood with the redox dye new methylene blue[8] Starch gel electrophoresis in Tris-EDTA-borate buffer, pH 8 6 (ref 9), revealed a faint abnormal haemoglobin band just anodic to HbA₂ (Fig 1) Mild, compensated, chronic haemolysis, slightly increased reticulocyte levels (2 0–5 7%) and the abnormal haemoglobin were also demonstrated in five relatives of the propositus (Table 1)

Hb Bushwick was purified by column chromatography on

Fig 1 Starch gel electrophoresis in Tris-EDTA-borate buffer, pH 8 6, of haemolysates from (AA) normal individual and (II-2) propositus with Hb Bushwick stained with Amido Black The anode is towards the top

Table 1 Haematological values of family members

Subject	Hct (%)	Hb (g%)	RBC (×10⁶ per µl)	MCV (µl)	MCH (pg)	MCHC (%)	Reticulocytes (%)	Abnormal electrophoresis	Inclusion bodies
Father (I-1)	40 5	13 1	4 23	96	31	32 4	3 6	+	+
Mother (I-2)	40 0	12 9	4 98	80	25 9	32 2	1 4	—	—
Propositus (II-2)	36 2(33 3)*	11.1(10.5)	3 76(3 42)	96(97)	30 1(31 6)	30 6(30 7)	4 1(15 0)	+	+
Sister (II-4)	36 0	12 2	3.91	92	31 0	34 8	3 3	+	
Brother (II-7)	46 0	16 6	5 58	82	29.8	36 1	1 6	—	
Son (III-1)	46 3	16 1	5 36	86	30 1	35 4	1 0	—	—
Son (III-2)	42 0	14 1	4 69	89	30.1	34 2	5 7	+	+
Nephew (III-4)	36.4	11 7	4.72	78	24 8	32	2 0	+	
Nephew (III-5)	35 5	12 1	3.89	91	31 1	34 1	3 1	+	

*Figures in parentheses represent values obtained in acute haemolytic episode

DEAE-cellulose in Tris-phosphate buffer, pH 8 6 (ref 10) When estimated by haem absorption at 540 nm, the proportion of Hb Bushwick in the haemolysate was 0.7–1 2% whereas the concentration of HbA_2 was 1 2–2 4% When the relative haem and protein contents of haemoglobin fractions prepared by chromatography were compared, however, the ratios of the parameters reflecting haem content (pyridine haemochromogen[11] and absorbance at 420 and 540 nm) to the parameters of protein concentration (absorbance at 280 nm and total nitrogen content), were one-third to one-half lower in the Hb Bushwick samples than in the HbA samples This suggests that Hb Bushwick is partially depleted of haem groups

Table 2 Amino-acid analysis of $\beta^{Bushwick}TpIX$

	$\beta^A TpIX$	$\beta_{Bushwick}TpIX$	Difference
Val	1	1 7 (2)	+1
Leu	4	3 9 (4)	
Gly	2	1 1 (1)	−1
Ala	2	2 1 (2)	
Phe	1	1 1 (1)	
Ser	1	0 9 (1)	
Asx	3	2 9 (3)	
His	1	0 9 (1)	
Lys	1	1 0 (1)	

The α and β chains of Hb Bushwick were separated by column chromatography on CM-cellulose in 8 M urea at pH 6 7 (ref 12) Both polypeptides eluted in the positions expected for the corresponding normal globin chains The two globin fractions were amino-ethylated, digested with trypsin and peptide maps prepared[12] The fingerprints of both chains of Hb Bushwick were identical to the patterns obtained with

the corresponding polypeptides derived from HbA All the peptides of both the α and β chains of Hb Bushwick were therefore subjected to amino-acid analysis The composition of $\beta^{Bushwick}TpIX$ (positions 67–82) indicated that there was one more valine residue and one less glycine residue than expected for $\beta^A TpIX$ (Table 2) In $\beta^A TpIX$, glycine occupies positions 69 and 74 When $\beta^{Bushwick}TpIX$ was digested with chymotrypsin the resulting peptide corresponding to positions 67–71 had a normal composition, whereas the chymotryptic peptide corresponding to positions 72–75 had the composition Ser, Asp, Val, Leu This result indicated that the substitution of valine for glycine is at position 74 in the β chain of Hb Bushwick

Glycine at β74 occupies position 18 in the E helix and faces inward towards the haem crevice in a crowded cleft which separates the E helix, the EF corner, and the F helix[13] Glycine is invariant in this position for all known β chains except the kangaroo[14] Introduction of an amino acid with a larger side chain into this tightly packed area would be expected to make the molecule unstable The insertion of the large valine residue into the scale model of haemoglobin at position β74 brings the γ carbons of valine closer than the minimal permissible van der Waals distance to several atoms in serine β70 (E14), threonine β84 (EF8) and leucine β82 (EF6) (M F Perutz, personal communication). These short contacts would force apart the E and F helices between which the haem group is suspended This widening of the haem pocket could weaken several haem–globin contacts which probably accounts for the loss of haem from Hb Bushwick Another human haemoglobin mutant, Hb Shepherds Bush, has a substitution of aspartic acid for glycine at β74 (ref 15) The side chain of aspartic acid is thought to be able to swing out of the haem pocket towards the surface avoiding complete disruption of

Table 3 Incorporation of ³H-leucine in haemoglobin by reticulocytes

Time (min)	Haemoglobin	Total radioactivity* (c p m)	Total radioactivity† (c p m) α	β	Specific activity c p m per mg*‡ α	β
15	A	91,800	4,647	17,521	58	198
	Bushwick	30,276	1,942	5,555	992	3,676
50	A	434,330	18,125	58,156	290	934
	Bushwick	111,720	6,781	8,165	6,576	8,760
5	A	82,464	13,150	17,128		
	Bushwick	33,191	2,795	6,916		
120	A	2,463,340	530,918	623,210		
	Bushwick	315,290	68,947	30,051		
5	A	65,219	25,476	39,743	140	243
	Bushwick	42,683	9,120	33,743	2,886	13,385
90	A	1,205,160	512,834	692,326	4,977	7,445
	Bushwick	294,252	196,168	98,084	73,660	45,280
5	Whole haemolysate		24,300	24,397		
90	Whole haemolysate		332,940	391,745		

*By DEAE cellulose chromatography
†By CM cellulose chromatography
‡Corrected for carrier protein,

he tertiary structure[15] Hb Shepherds Bush, which comprises 24% of the haemoglobin in the blood of affected subjects, may thus be more stable than Hb Bushwick

Because only an unusually small amount of Hb Bushwick could be demonstrated by haemoglobin electrophoresis or chromatography, attempts were made to detect the presence of additional amounts of β Bushwick in erythrocytes by amino-acid analysis Since β^ATpIX has a glycine-alanine ratio of 1 0 whereas that of $\beta^{Bushwick}$TpIX is 0 5, βTpIX prepared from an unfractionated haemolysate would have an intermediate Gly–Ala ratio depending on the proportions of the two haemoglobins present Analysis of peptide βTpIX of HbA purified from the haemolysate of the proposita revealed a Gly–Ala ratio of 1 0, suggesting that no Hb Bushwick was contaminating that fraction Three analyses of peptide βTpIX obtained from whole globin prepared by direct acid–acetone treatment of the unfractionated haemolysate, including membranes, gave Gly–Ala ratios of 0 97, 0 93 and 0 92, indicating that the erythrocytes could contain as much as 5–15% of Hb Bushwick Denaturation of the unstable haemoglobin or binding to cell membranes during preparation of a haemolysate could explain the lower amounts of Hb Bushwick demonstrable by chromatography or electrophoresis

The relative synthetic rates of HbA and Hb Bushwick were directly examined in vitro Washed erythrocytes from the propositus were incubated for 5–120 min with ³H-leucine The cells were lysed and HbA and Hb Bushwick were separated by DEAE chromatography The radioactivity in each haemoglobin fraction was measured (Table 3) and globin was prepared The α and β chains were separated and the total radioactivity and specific activity of the fractions were determined (Table 3) After incubation for 5–15 min the total radioactivity incorporated into β^A was only 1 2–3 times that in $\beta^{Bushwick}$ This indicated that the two β globin chains were synthesised in grossly equivalent amounts and suggested that the low proportion of Hb Bushwick found in blood was a result of postsynthetic loss of the unstable variant This rapid loss of newly synthesised Hb Bushwick was confirmed by the steep rise in the ratio of the radioactivity in β^A and $\beta^{Bushwick}$ to 7–20 1 after 50–120 min incubation A pool of non-radioactive excess free α chains in the erythrocytes was suggested by the low specific activity of the α chains relative to β chains in both haemoglobins after short periods of incubation The excess α chains were a result of degradation of preformed Hb Bushwick and were not the result of a deficit in β globin synthesis Synthesis of α and β globin was balanced in an experiment in which the entire haemolysate, including membranes, was converted directly to globin before chain separation (Table 3)

In spite of the evidence of rapid postsynthetic loss of Hb Bushwick, the erythrocytes did not seem hypochromic when examined microscopically and exhibited a normal mean corpuscular haemoglobin of 31 pg (Table 1) These findings suggest that the normal trans β^A allele was able to compensate for the loss of Hb Bushwick by increasing β^A globin synthesis There are several other examples of subjects having unstable β chain variants in amounts less than 20% of the total circulating haemoglobin[16-23] In all reports, except one[16], the erythrocyte mean corpuscular haemoglobin was normal, or nearly so, and the red cells appeared normochromic when examined under the microscope Regardless of whether the low intracellular concentrations of these unstable haemoglobins was primarily a result of increased destruction or decreased synthesis, a compensatory increase in β^A synthesis directed by the trans allele had to occur Such a degree of compensation is lacking in the β-thalassaemia trait[9] where a defect in one of two β globin alleles is associated with a decreased cellular content of β globin mRNA (ref 24), decreased HbA production and a low mean corpuscular haemoglobin content[9] The trans β^A gene thus does not seem to be able to compensate by increasing its gene product in thalassaemic cells as effectively as in cells with the unstable haemoglobins Possible mechanisms

for such failure of compensation in thalassaemia, include the presence of an inhibitor of β-globin synthesis or the lack of a synthesis-promoting substance These mechanisms have been postulated previously[25,26] but confirmation has been lacking It would seem worth while to continue exploration in this area

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High cooperativity of haemoglobin M Boston in the completely reduced state

HAEMOGLOBIN (Hb) M Boston and Hb M Iwate, in which distal and proximal histidines, respectively, in the haem pockets of α chains are replaced by tyrosines, bind ligands only to the normal β chains, and have α chains stabilised in the ferric form These M Hbs contain mutant α chains and have abnormally low oxygen affinity, almost no Bohr effect, and virtually no haem–haem interaction[1,2] The β chain mutants, Hb M Hyde Park and M Saskatoon, have normal oxygen affinity and a
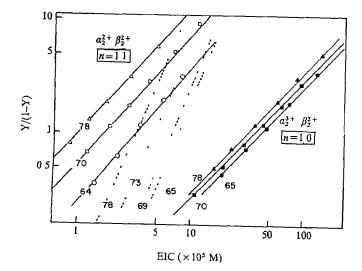
normal Bohr effect but show almost no haem–haem inter-action[3,4] Thus among the M Hbs, the proximal and distal mutants of the same chain display similar ligand binding properties When abnormal α chains of Hb M Iwate are completely reduced using dithionite, the affinity for CO and Bohr effect were almost normal, but Hill's constant was still one[1] It has been suggested that the ligand binding properties of completely reduced Hb M Boston are approximately the same as those of Hb M Iwate[5,6] We have studied the equilibria of Hb M Boston and Hb M Iwate in both the half ferric and completely reduced states using ethylisocyanide (EIC), a non-oxidising ligand which equilibrates rapidly with haemoglobin, and here report that the completely reduced Hb M Boston shows ligand binding properties similar to those of Hb A.

Hb M Boston and Hb M Iwate were separated from Hb A using an Amberlite CG-50 column (1 5×40 cm) previously equilibrated with 0 05 M phosphate buffer, pH 7 0 After Hb A had been washed out with 2 1 of the same buffer, Hb M remaining on the column was eluted using a linear gradient from 0 05 to 0 2 M phosphate buffer (pH 7 0) with 300 ml of each buffer Purity was examined by starch gel electrophoresis of methaemoglobin M, prepared by oxidation of the sample with potassium ferricyanide

Completely reduced Hb M was prepared by bubbling with O gas (helium–isobutane, 99.05 . 0 95), subsequent addition of 3 mg ml^{-1} sodium dithionite and standing overnight at room temperature in a cell with a light path of 1 cm and a rubber stopper seal Half ferric Hb M Iwate, in which normal β chains contain ferrous haems and abnormal α chains ferric haems, was prepared by deoxygenation of the sample solution and the addition of sodium ascorbate to give a final concentration of 5 mM Half ferric Hb M Boston was prepared as follows Completely reduced Hb M Boston was separated from dithionite by passage through a Sephadex G-25 column, and abnormal α chains were oxidised using dissolved oxygen for 20 min at room temperature Absorption spectra of half ferric Hb M Iwate and Hb M Boston showed that only abnormal α chains were ferric Equilibrium with EIC was determined spectrophotometrically following the method of Anderson *et al*[7].

The EIC dissociation curves for Hb M Iwate at various pH are shown in Fig 1 In the half-ferric state, CO affinity was very low and the Bohr effect very small When abnormal α chains were reduced and all haems in Hb M Iwate were capable of binding EIC, affinity was a little greater than that of Hb A and

Fig. 1 EIC equilibrium curves of Hb M Iwate in 0 2 M phosphate buffer at 25° C The fractional saturation of haemoglobin with EIC, Y, against the EIC concentration, c, is plotted according to a modified form of Hill's equation, $\log Y/(1-Y) = n\log c + \log K$ Open and filled symbols represent the experimental points for the completely reduced and the half ferric state, respectively ———, Hb M, - - - -, Hb A pHs shown beside plots
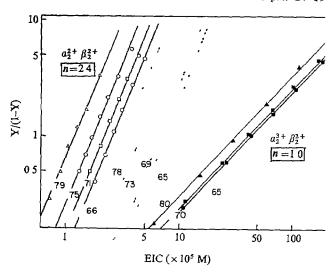

the magnitude of Bohr effect was almost normal Hill's constant however, was approximately one in both the states The results are qualitatively similar to those obtained for C binding to Hb M Iwate[1] EIC equilibrium curves for half ferric Hb M Boston were almost the same as those for Hb M Iwa (Fig 2) Completely reduced Hb M Boston, however, showe haem–haem interaction as strong as Hb A, although its affinit and Bohr effect were similar to those of completely reduce Hb M Iwate

X-ray analysis of Hb M Boston shows that the ferric iro atoms in the abnormal α chains are bonded to the phenolat side chains of the tyrosines which have replaced the dista histidines[6] The α haem groups of Hb M Iwate are probabl bonded to both the distal histidines and proximal tyrosines[6] The resulting changes in tertiary structure of the α-chain mutants are considered to stabilise the haemoglobin tetrame in the quaternary T structure, which causes the abnorma ligand binding properties[5,6] When the iron atoms of th α haems of Hb M Boston are reduced by dithionite, bond between the haem irons and the phenolates may be broken an bonds with the proximal histidines may be formed The resulting structure seems to be similar to that of Hb A as judged from th EIC binding properties Similarly, reduction of ferric irons in abnormal α chains of Hb M Iwate may break bonds betweer α haem irons and distal histidines, which increases both th ligand affinity and Bohr effect Replacement of proxima histidine by tyrosine in Hb M Iwate, however, abolishes th cooperativity in EIC binding, the importance of the proxima histidine in cooperative ligand binding is suggested

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Fig. 2 EIC equilibrium curves for Hb M Boston Experimental conditions and symbols used are as in Fig 1
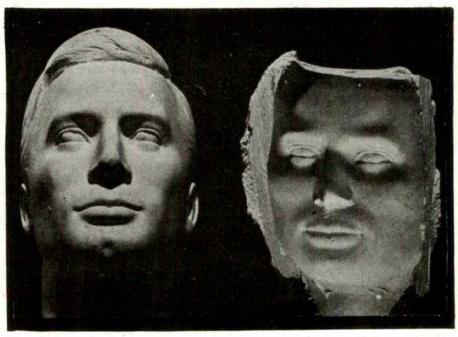
reviews

How man has seen the world

THE declared aim of this ambitious handbook* is "to bring together essential aspects of the very large, diverse and widely scattered literature on human perception." The present volumes are the first of 12; a third volume, *Biology of Perceptual Systems,* was published in 1973. Volumes 4–6 will cover the individual senses; and in the last six volumes it is planned to include areas such as speech and language, aesthetics, the perception of form, of space and of objects, and the perceptual aspects of thought.

Yet this is not a handbook. It is a very large collection of essays. There are many notable chapters by individual contributors, but the sum of the parts cannot fairly be represented as a true handbook. Cross-references within and between volumes are almost completely absent and there is much internal evidence to suggest that contributors have not read each other's chapters: thus, R. M. Boynton, who gives an account in Volume 1 of J. J. Gibson's theory of visual perception, cannot have been invited to read those passages in the same volume in which M. Wertheimer makes the elementary mistake of speaking of visual receptors firing impulses.

Indeed, there is grave doubt as to whether the editors themselves have read all the contributions. Very often the same ground is covered two or three times without comment or cross-reference, so that the reader is left wondering whether he is missing some subtlety; there seem, for example, to be three introductions to epistemology, two introductions to psychophysics and two accounts of multidimensional scaling. The very sequence of chapters is sometimes bizarre: a chapter by J. R.

Handbook of Perception. Vol. 1: Historical and Philosophical Roots of Perception. Pp. xix+431. $23.50; £11.30. Vol. 2: *Psychological Judgement and Measurement.* Pp. xix+556. $29.50; £14.15. Edited by Edward C. Carterette and Morton P. Friedman. (Academic: New York and London, 1974.)
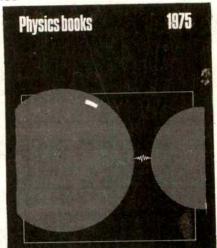
Royce on epistemological psychology appears, inexplicably, in the historical section of Volume 1, whereas, if it belonged anywhere in that volume, it would have been as a bridge between the philosophical and the psychological sections. Similarly, in Volume 2, a relatively technical paper by A. Sandusky on memory effects in psychophysics is separated from A. Parducci's paper on contextual effects and is instead inserted between a short and very readable introduction to psychophysics by F. Nowell Jones and a long and very readable introduction to psychophysics by E. Galanter. In short, in spite of the 2 editors and 17 members of the editorial board, almost the only evidence of an editorial hand is the use throughout of *cf* where *v* is properly required.

The editors must, however, enjoy some credit for their choice of contributors. Many of the individual chapters are of a very high standard, particularly Hochberg's excellent review of the history and present state of the Gestalt tradition; Berlyne's introduction to attention; Garner's introduction to attention; and the comprehensive account of multidimensional scaling by Carroll and Wish. Luce and Green combine to write a supplement to the 1966 book on signal detection theory by Green and Swets. R. L. Gregory, in a section of Volume 1 devoted to grand

Though both are viewed under the same lighting and have the same texture, the face on the right does not appear as a mould of that on the left.

theories of perception, does what the editors have failed to do and contrasts different theoretical approaches, considering the capacity of each to accommodate a number of facts that he has carefully chosen to support his own view that "perceptions are hypotheses". Elsewhere in the same volume there is a critique of this class of theory by Metzger but, characteristically, there are no cross-references between his chapter and those by Gregory and Hochberg.

Although Volume 1 is entitled *Historical and Philosophical Roots of Perception,* the history of perceptual theory is covered very inadequately. The origins of the sensory sciences in physics and medicine are almost completely neglected, as is the history of the study of senses other than vision. Such history as is dealt with reads soundly, but it is often drawn from secondary sources and professional historians of science would judge it presentist and Whiggish. In recent years historians of science have notoriously drawn upon psychological illustrations of the hypothetical nature of perception and thus it is the more unhappily ironic that this opportunity has not been taken to introduce a more hermeneutic approach to the history of perceptual theory and to discuss theories of perception in the context of the way man has seen the world.
J. D. Mollon

Physics books 1975

Acoustics 1974

The Invited Lectures Presented at the Eighth International Congress on Acoustics London 1974

Edited by R.W.B. STEPHENS

February 1975 : 220 pages : illustrated
412 12400 9 : hardback : £5.00

The emphasis in many of the lectures in this book is on environmental acoustics.

The objective is to review the present state of knowledge of the principal sources and propagation of noise, the prospects for its reduction, the role of building acoustics in noise control, and the psychological and biological effect of sound on man.

Acoustics and Vibration Progress Volume 1

Edited by R.W.B. STEPHENS and H.G. LEVENTHALL

November 1974 : 254 pages : illustrated
412 11560 3 : hardback : £6.00

This volume contains reviews on traffic noise, chemical ultrasonics, ambient noise, acoustic emission, and vibration and noise in building structures.

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Model standard for hydrogen bonding

Hydrogen Bonding. By Melvin D. Joesten and L. J. Schaad. Pp. vi+622. (Marcel Dekker: New York, 1974.) $45.00.

THIS book is explicitly planned as supplemental to Pimentel and McClellan's *Hydrogen Bond,* " . . . to provide comprehensive coverage of those topics which account for a large percentage of the hydrogen bonding (HB) papers published since . . . (1960)". It reaches a standard not unworthy of its model.

Four of the five excellent chapters are by Professor Joesten, and cover the detection of HB, thermodynamics and kinetics, (spectroscopic) correlations, and intramolecular and homo-intermolecular HB. The other chapter, by Professor Schaad, deals with the theory of HB exceedingly well; with minimal mathematics, he surveys the application of quantum mechanics to molecules, carefully explains the types of approximation necessary with HB, and assesses their limitations—all this with masterly elegance. He ends his chapter with a list of theoretical studies of HB systems carried out between 1960 and 1973. He

writes: "It is tempting for the expe mentalist to conclude that since f theoretical predictions are complet certain one can ignore all theoreti results. The difficult alternative weighing each separately is proba more useful".

Besides some 250 pages of strai text, there are 130 pages of tabula results and 2,703 references. The bc is largely concerned with spect scopic studies and their thermodynar consequences. This is the way most the current HB literature runs; implies no adverse criticism to sta that this book concentrates on re ively weak HB; that it has less to about strong HB or its study by diff tion methods. The book is an exam of the pleasing results that are sor times achieved by photo-offset. The are few errors—something is aw with the legend to Fig. 5.1. The pr will deter most individuals from buy their own copy; but perhaps these d even 4 pence a page is not preposter for so valuable a book.

J. C. Speakm

Limited horizons

Television review by Robbie Vickers

THE BBC are to be commended in launching *Choices for Tomorrow*, a new series of half-hour programmes on the environment and how we use or misuse it. The first, *Woodland or Wasteland* (Monday April 7; 11.05 p.m.) went only part of the way to solving the problems facing educational television.

Michael Pye of the *Sunday Times,* aired his own personal views on the trials and tribulations of the tree in Britain—government forestry policy, recycling of paper products, and so on. His impressions—they were essentially his, and on a topic very much related to his own sphere of interest—were interesting and should provoke thought and discussion.

The comments on the way we are likely to affect the world we live in were, however, left too open-ended, making the whole programme look very much like a miniature and limited *Horizon.* the time available, a 'chat' programme this sort cannot attempt to supply itive answers—and we wouldn't war this—but it could forward suggestons; it is simply not constructive never explicitly to state alternatives. Such comments as "every time I write an article, the paper publishes one and a quarter million copies of it and that means 4,000 trees die"— do not get us anywhere other than making us aware of the problems.

Before making suggestions, however,

perhaps we should get our facts straig At the moment, the cost of de-inki and bleaching paper far outweighs benefits that may accrue from its re-us It is not until a balance is reached th this method of conserving a resour can become viable and economical, though the organised collection of wa paper is a method worth investigati on a commercial scale.

For some time now, the Forest Commission have, as a matter of h priority, considered the integration maximum wood production and amenity value or aesthetic value of c woodlands. National Parks were c ceived to meet this need, at a ti when the idea of increased leisure tir came to the fore. To some peop therefore, Mr Pye's ideas may ha been misleading, particularly at such late hour in the evening.

What was given was a popularise one-sided, slightly inaccurate vie at least there was sufficient comedy ar wit to sustain enough interest for peop to take the punch-lines, but people ne easily accessible informed comment make reasoned judgements about t world in which they live.

Allowing for the limitations and personal nature of the views express with a bigger budget, more time a research staff, this programme cou become worth a slot during peak vie ing hours. Future programmes will co sider the energy shortage, polluti control and the effects buildings ha on our behaviour.